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ERRATA AND AUTHORS' EMENDATIONS

Pages 60–63, legends to figures 3–8, "phosphate" should read "phosphorus."

Page 175, lines 45–46, Mr. Ainslie informs the author that he has never succeeded in rearing *Pyrausta ainsliei* to maturity on *Nelumbo lutea* as here stated. In the discussion of *P. ainsliei* it should be stated that the old name *P. obumbratalis* Lederer may eventually prove to apply to this species. Without an examination of the genitalia of Lederer's types in Europe, however, it can not be determined whether his name applies to this species or to *P. penitalis* Grote, if to either. In the meantime it is safest to employ a definite name to a definite concept.

Page 333, Table III, column 8, next to last line, and column 9, next to last line, "3.329" should read "3.320."

Page 335, Table VI, "phosphates" should read "phosphate."

Page 339, Table XIII, column 5, line 8, "92" should read "80."

Page 393, line 27, "in part by weight" should read "in parts by weight."

Page 394, Table I, column 9, line 10, "54.5" should read "5.45." Note *a*, "expressed in parts" should read "expressed in parts by weight."

Page 395, Table II, continued, column 6, line 4, "1 : 1.83" should read "1 : 0.83." Second part of table, column 6, lines 1 and 2, "1 : 1.05" and "1 : 1.37" should be transposed. Line 10 of text, "basal ratio" should read "basal ration."

Page 445, line 15 of text, "from 13.3 larvæ to each fruit in 1916 to 20.3 in 1917 and to 34.6 in 1918" should read "from 13.3 per cent in 1916 to 20.3 per cent in 1917 and to 34.6 per cent in 1918."

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NOTES ON THE COMPOSITION OF THE SORGHUM PLANT[1]

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I.—INTRODUCTION

Since 1877, when the United States Department of Agriculture undertook the investigation of sorghum (*Sorghum vulgare*) as a source of crystallized sugar, many thousands of analyses of sorghum juice, from many different varieties, have been made and published. As a result, there is a well-established fund of knowledge concerning the kinds and quantities of sugars in the juice, especially for the more temperate regions of the United States. Considerable work has been done in this and in other countries on the effect of removing the seed heads on the composition of the juice. Also a little work has been done on the practices followed in the manufacture of sorghum sirup. However, when one of the present writers, R. M. West, undertook in 1912 to place the sorghum industry in Minnesota on a better economic and scientific basis, the need for further chemical investigations was seen at once. It was apparent (1) that, considering the effect of climatic factors on the composition of the cane, more exact knowledge was needed concerning the behavior of sorghum grown in the most northern limit of its range; (2) that the utilization of the cane somewhat prior to maturity, and very often after being killed by frost, would be necessary in order to lengthen the milling season as much as possible; (3) that the methods of defecation and evaporation in vogue were decidedly in need of improvement and standardization; (4) that for economic reasons the small-scale manufacture of sorghum sirup, with inefficient mills, little or no defecation, and slow boiling, would have to give way to large-scale production or the rapid decrease in production of sirup, as witnessed for the last thirty years, would no doubt continue. The investigations at this Station resulted in the accumulation of considerable data of both scientific and practical interest. The

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latter have been compiled in a Station bulletin; [1] the former are set forth in the present paper.

II.—METHOD OF INVESTIGATION

VARIETY TESTS.—Several varieties of cane grown on University Farm, St. Paul, were tested for several consecutive years to ascertain their behavior in this region.[2] Analytical studies were made on but three varieties, however—Minnesota Early Amber, an old, very well-established, and almost universally grown variety not only in Minnesota but in the whole country; Early Rose, a variety isolated from Early Amber in southern Minnesota about 15 years ago; and Dakota Amber, another selection from Early Amber that was made in South Dakota. The data in this paper are the averaged results from these three varieties.

SAMPLES.—The data on the progressive changes in composition of the cane cover a period of three growing seasons. Not all data, however, were obtained for all the years. The stages of growth at which samples were taken were as follows:

(1) When the panicles first appeared.

(2) When the panicles were wholly emerged.

(3) When the anthers of the blossoms appeared at the middle of the panicles.

(4) When the panicles were in full bloom.

(5) When the seed was in milk.

(6) When the seed was in soft dough.

(7) When the seed was in hard dough.

(8) When the seed was brittle and mature.

During these stages the plants increased in height by 2 or 3 feet.

In taking samples, 6 to 10 plants were selected which were of nearly the same stage of growth and which at the same time were as representative as possible of the plot. Two samples were cut from each plot at each stage of growth. One was weighed, sacked, and dried; the other was weighed, stripped, topped, and the juice extracted by pressing with a small 3-roll power mill.

ANALYSIS.—The leaves, tops, cane, juice, and bagasse were weighed separately and the weights recorded, together with the losses in weight during stripping and pressing. The leaves, tops, and bagasse were sacked separately and, together with the sample of the whole plant, dried as rapidly as possible in a steam oven to less than air-dry moisture content. The samples were then exposed to the air of a well-ventilated room until there was no further increase in weight due to absorption of moisture. The finely ground air-dried material was resampled and the usual proximate determinations were made, together with an analysis of the ash for

[1] WILLAMAN, J. J., WEST, R. M., and BULL, C. P. SORGHUM AND SORGHUM-SIRUP MANUFACTURE. Minn. Agr. Exp. Sta. Bul. 187.

[2] Acknowledgments are due to Prof. C. P. Bull, of this Station, for the agronomic phases of this work.

the percentage of potash and phosphoric acid. For these determinations the official methods (*20*) [1] were followed, except that crude fiber was determined by the modified Sweeney (*10*) method, and the preparation of the sample for the potash and phosphoric acid was accomplished by the modified wet ignition method (*18*).

III.—PROPORTION OF LEAVES AND TOPS TO CANES

Since cane is brought to the mills in various conditions, such as fresh whole cane with or without seed heads or leaves or both, and partially dried cane with or without tops and leaves, it is desirable that the average proportion of these three parts be known by the mill operator in computing the value of the various grades of cane. Table I presents the average figures for six plots in 1913.

TABLE I.—*Relative percentage of leaves, seed heads, and clean cane in whole cane when fresh and in whole cane when partially dried*

Stage of growth.	Whole cane, fresh.			Whole cane, partially dried.[1]		
	Leaves.	Tops.	Clean cane.	Leaves.	Tops.	Clean cane.
Panicles appearing	19.8	8.4	71.8	.....	.....	.....
Panicles out	17.2	8.4	74.4	.....	.....	.....
Blossoms appearing	15.8	8.6	75.6	.....	.....	.....
Full bloom	15.2	8.2	76.6	.....	.....	.....
Seed in milk	16.5	11.1	72.4	.....	.....	.....
Seed in dough	15.3	14.1	70.6	.....	.....	.....
Seed dry	16.0	16.2	67.8	10.0	11.0	79.0
Seed mature	14.9	16.7	68.4	9.0	10.0	81.0

[1] Computed from various field data.

Collier (*5, p. 142*) reports 72 per cent of clean cane from fresh material. He also says that the leaves constitute 15 per cent of the topped stalks. This figure appears rather high, for it is practically the percentage of leaves in the whole cane as shown in the above table. From the above data the writers were able to construct a table for the use of manufacturers, by which they could compute the value of a ton of cane according to its condition when weighed at the factory.[2]

IV.—PROXIMATE COMPOSITION OF THE PLANT

Many analyses are on record of the proximate constituents of the various parts of the sorghum plant, designed to show its feeding value. Most of them agree substantially with the data obtained in the present work, at least as regards the general trend of development of the various constitutents. It would be futile to review these analyses. The present data have been calculated in various ways in order to reveal facts not

[1] Reference is made by number (italic) to Literature cited, p. 30–31.

[2] WILLAMAN, J. J., WEST, R. M., and BULL, C. P. OP CIT.

hitherto pointed out concerning the physiology of this plant and the relations between the various parts of the plant as maturity is approached. It is well to keep in mind, while perusing the graphs and data, that the sorghum plant, so far as we are concerned in the present investigations, is cultivated primarily for its sugar content. The data repeatedly reveal the subservience of all other constituents to the sugars. This same phenomenon has been pointed out for many other plants which specialize in the production of some one class of substances, as the starch in potato tubers and in corn seed, the sugars in fruits, the oil in flax, peanuts, and soybeans, and the sucrose in sugar beets and in sugar cane.

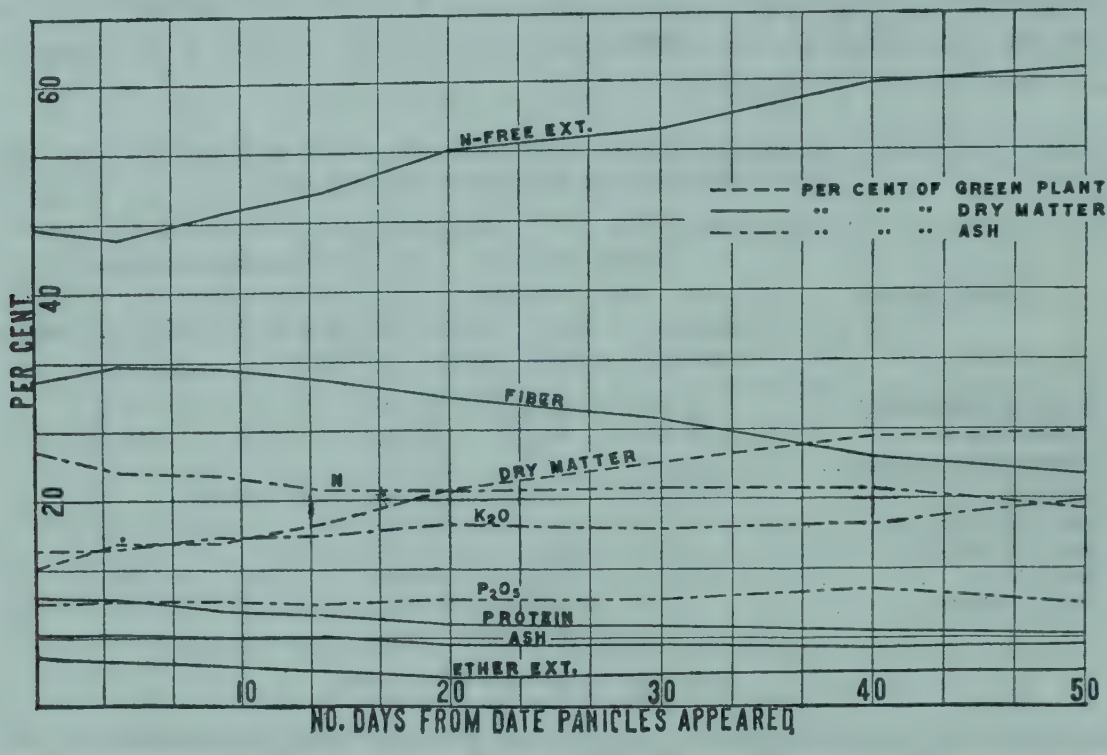

FIG. 1.—Development of the proximate and mineral constituents of the sorghum plant in the later stages of growth.

The results of the analytical studies are presented only in graphs, since their presentation in tables adds nothing to what is given in the charts.

Figure 1 shows the composition of the whole plant, expressed as percentages of the green plant and of the dry matter. The ash constituents are expressed as percentages of the total ash. The nitrogen content is computed to the ash basis, so as to make it comparable to the phosphorus and potash. There is a gradual increase in dry matter from about 12 per cent to about 26 per cent. Of this dry matter, the most prominent constituents are the fiber and the nitrogen-free extract. The latter is mostly starch, dextrose, and levulose in the younger stages, and mostly sucrose in the older. The percentages of fiber and of the other carbohydrates undergo progressive changes which are almost exactly equal to each other but opposite in character—that is, the percentage of

decrease of the fiber is the same as the percentage of increase of the soluble carbohydrates. The percentages of crude protein, ash, and ether extract remain practically the same throughout growth.

When these percentages are computed to the absolute weight of each constituent in one plant, the same facts, with but slight modification, are apparent. Figure 2 presents these data. The actual weights of protein, fat, and ash in each plant increase but slightly during the periods of growth studied; the fiber about doubles in weight, while the nitrogen-free extract trebles in weight. The stages of development studied here represent not only maturation of the plant but actual growth, the height increasing by 2 or 3 feet during these stages. It is apparent that the plant absorbs practically all of its mineral requirements, including nitrogen, during the early stages of growth, that it also lays down the necessary structures of protein and fiber during these stages, and that during the final maturation periods all the energies of the plant are directed toward the filling out of the seed and the storing of sugar in the cells of the cane. This program of development may prove to be the rule in all plants, as it has already been proved in several of them, notably in wheat, by Thatcher (*16*).

The sharp decline in the curves in figure 2 for the last growth period is explained by the fact that the plots had been culled of the larger plants and smaller plants had to be chosen for the final stage. Thus the percentage curves continue in the direction anticipated, while the curves of absolute weights show a declination.

The above observations concern the whole plant. Considering now the separate parts of the plant, it is found on examination of figure 3 that the leaves undergo changes in composition which are in many ways similar to those of the whole plant. The nitrogen-free extract exhibits a marked increase during the later stages. This is not paralleled by an equal decrease of fiber, however, as was the case with the whole plant. The fiber remains constant, both relatively and absolutely (fig. 4). The percentage of protein undergoes an appreciable decrease, while the absolute weight of it remains practically constant. The changes in dry matter are closely parallel to those of the nitrogen-free extract. This is corroborated by Collier's analyses of the juice of leaves (*5, p. 142*), which showed a considerable increase of sugars in the more mature stages. These data would indicate that the more mature the leaves the higher their feeding value.

Figures 5 and 6 present the curves for the composition of the tops. In the preparation of the samples the cane was cut off just below the lowest stem of the seed head, and the whole head used in the analyses. This of course resulted in the earlier samples' consisting mostly of stems, hence the high fiber content. Later, due to the filling out of the seeds with starch, the percentage of fiber underwent a marked decrease, while

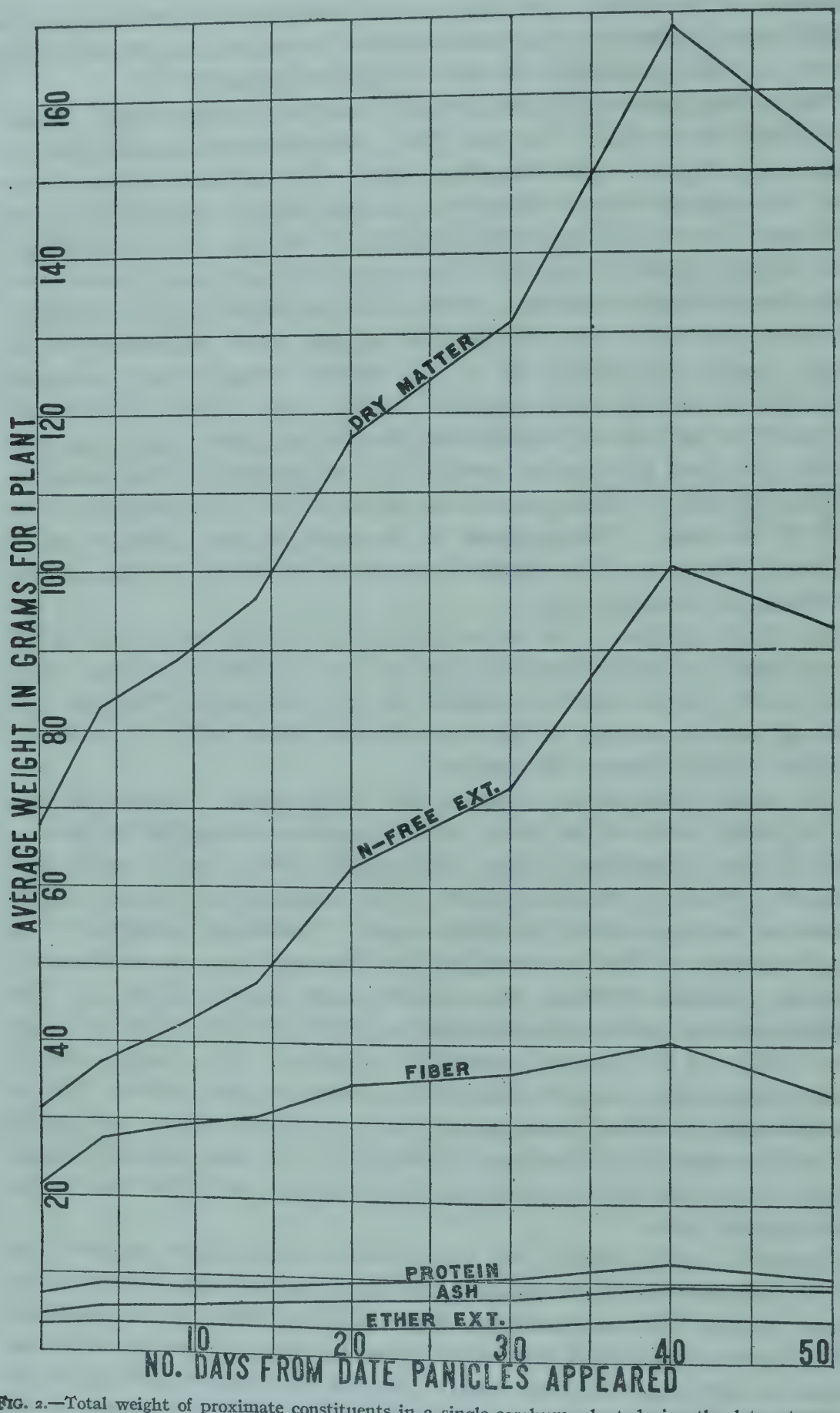

FIG. 2.—Total weight of proximate constituents in a single sorghum plant during the later stages of growth.

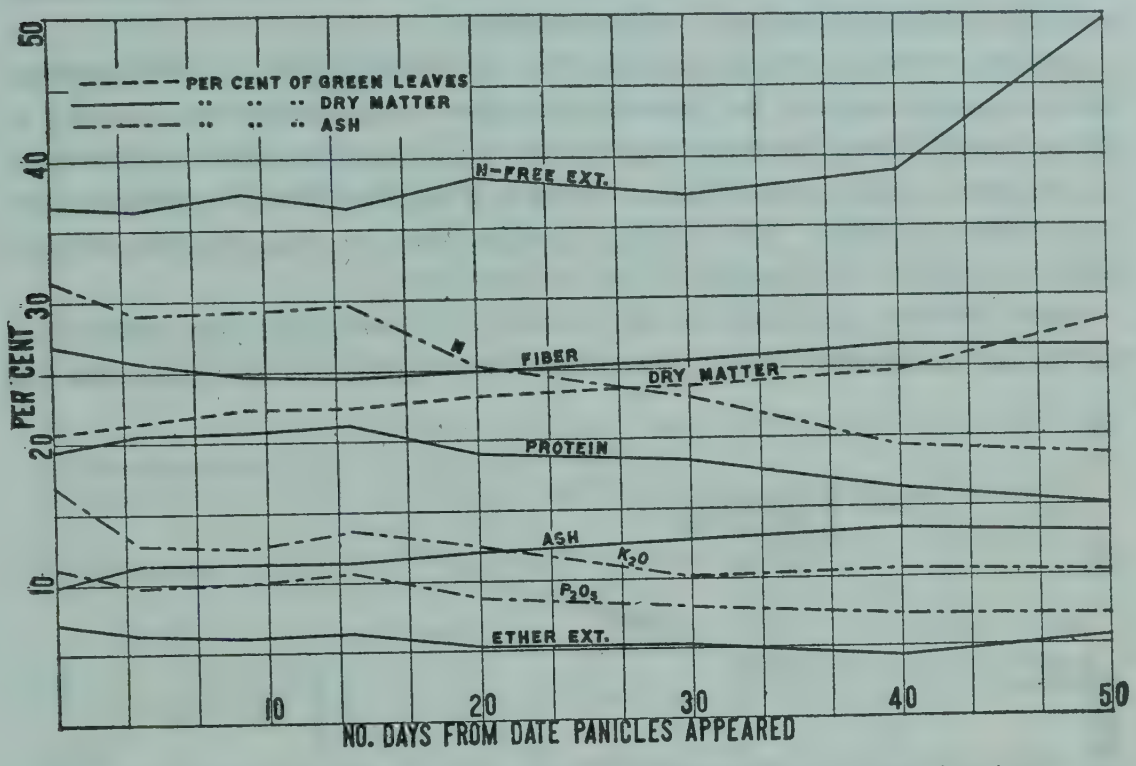

FIG. 3.—Development of the proximate and mineral constituents of the leaves of sorghum.

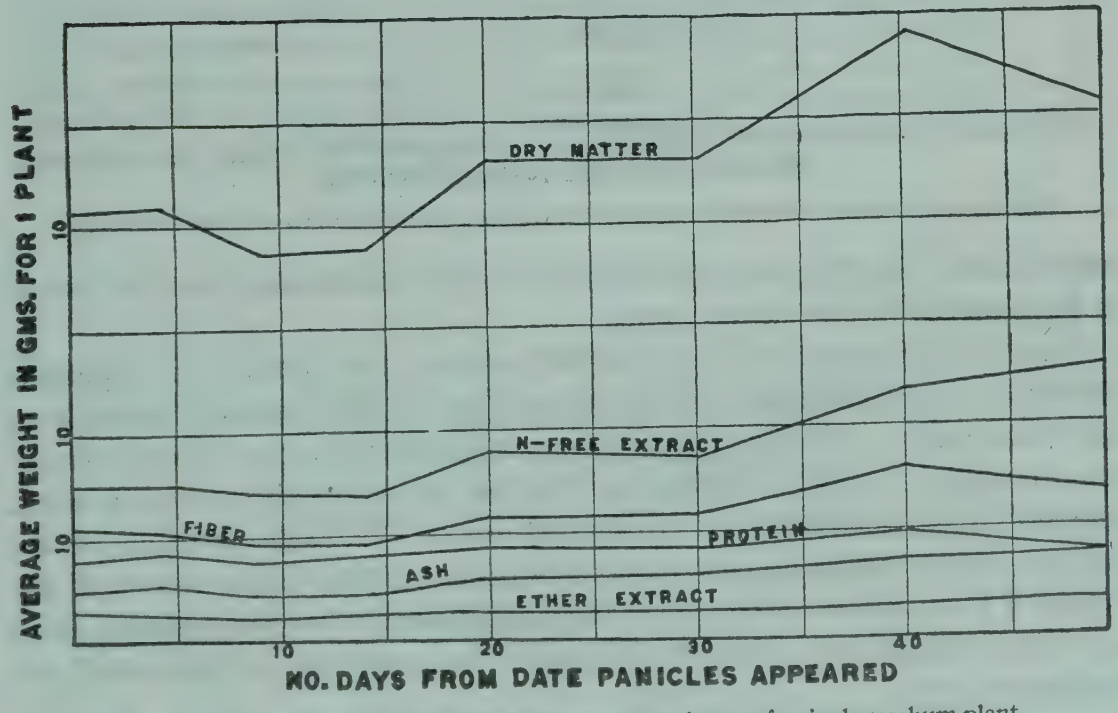

FIG. 4.—Total weight of proximate constituents in the leaves of a single sorghum plant.

its absolute amount remained nearly constant. The dry matter and the nitrogen-free extract again parallelled each other, while the protein, ash, and ether extract remained rather constant.

Figures 7 and 8 contain graphs for the composition of the bagasse. Great reliance can not be placed upon these data, since the amount of juice obtained from the cane varied from 34.5 to 37 per cent of the weight of the cane. The bagasse as analyzed thus contained a considerable proportion of juice. The relative proportions of the various constituents are similar to those of the leaves and tops.

Some interesting relations can be seen by collecting the percentage curves for individual constituents for various parts of the plant on the

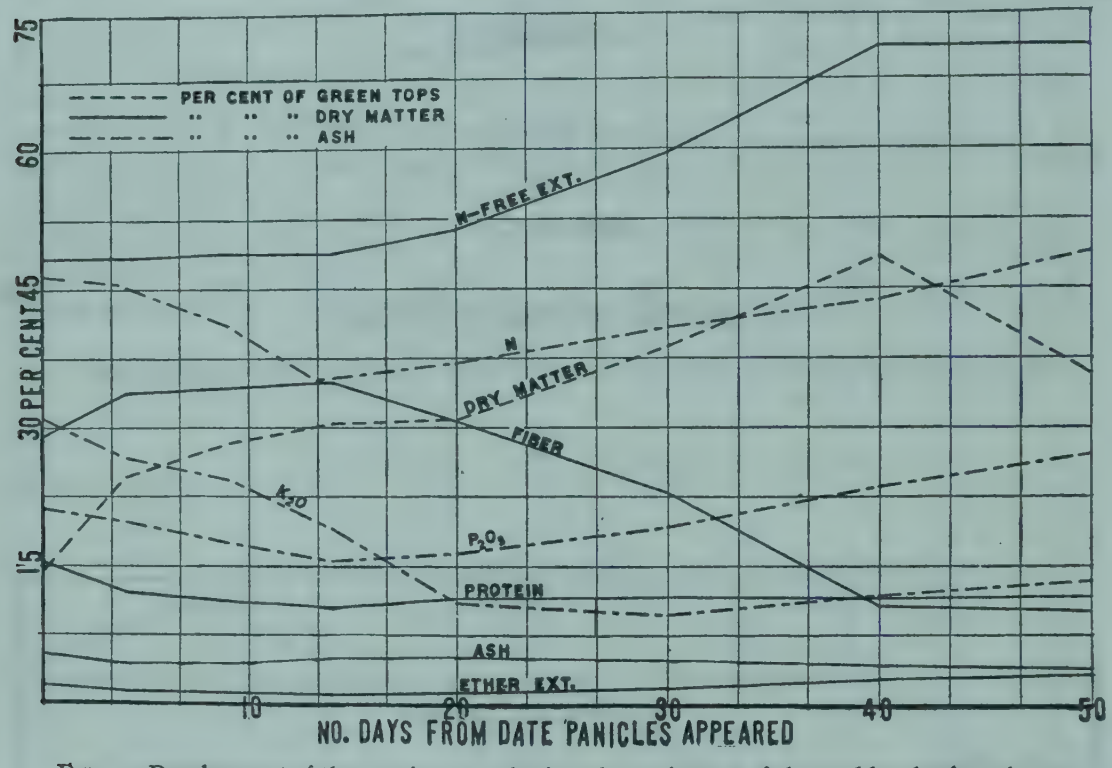

FIG. 5.—Development of the proximate and mineral constituents of the seed heads of sorghum.

same chart. In this way the history of each constituent can be viewed in its relation to the whole plant.

Figure 9 contains the dry matter curves. As would be expected, there is a general increase in the percentage of dry matter throughout the whole plant. The most marked increase is in the tops. The apparent abnormality of a sharp decrease in the last period of this curve is unexplained; it is no doubt an analytical error. A large portion of the increase in dry matter of the bagasse is probably due to the sugars of the retained juice, since the two curves parallel each other very closely, and since only about two-fifths of the juice was expressed by the small experimental mill.

Figure 10 contains the curves for the crude protein. As has been pointed out above, there is not only a regular decline in the percentage

of protein throughout the plant but the absolute weight of protein in each plant remains practically constant through the stages of growth studied here. This is no doubt brought about by the great increase in nitrogen-free extract sugars in the cane and starch in the seed heads.

Figure 11 contains the ether extract curves. They indicate that in all parts of the plant except the tops the ether extract remains constant. In the tops there is a slight increase. This constituent is not prominent

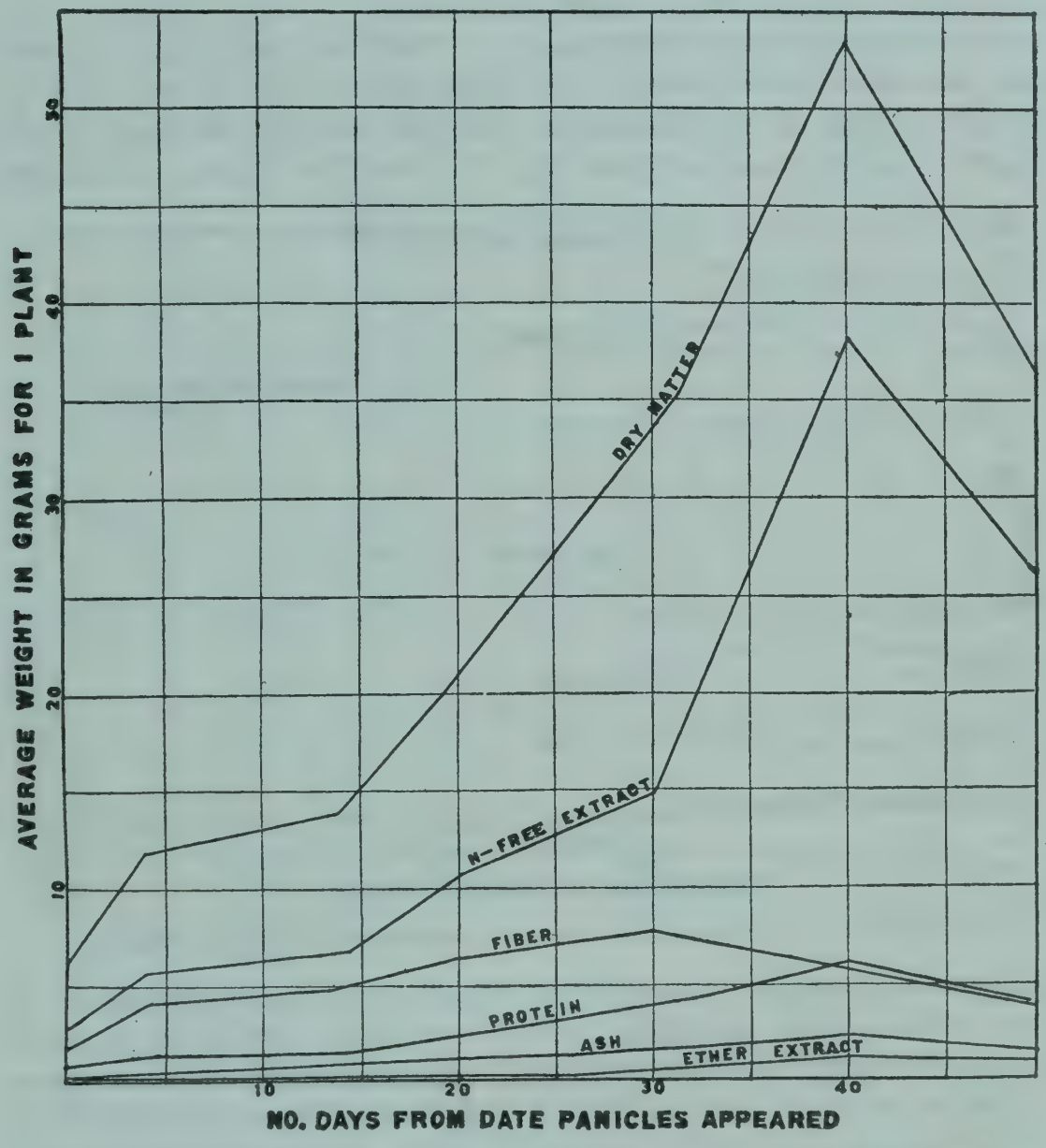

FIG. 6.—Total weight of proximate constituents in the seed head of a single sorghum plant.

in any part of the plant. The higher percentages in the leaves are due in large part to the chlorophyll.

The crude fiber curves are shown in figure 12. The curves for the bagasse and for the leaves are practically horizontal. The very sharp decrease in the fiber in the tops causes a marked decrease in the percentage of fiber in the whole plant also.

The nitrogen-free extract is the proximate constituent most characteristic of the sorghum plant and the constituent for which the plant is grown. The curves for this extract in the various parts of the plant are assembled in figure 13. There is a pronounced increase in all parts, most noticeable in the tops. Although the percentage of increase of nitrogen-free extract is greatest in the tops, the absolute increase is almost equally great in the juice because of the accumulation of sugars. This fact is brought out in figures 5, 7, and 8.

The ash curves are given in figure 14. There is a very apparent tendency for the mineral material to accumulate in the leaves. This is shown not only on the percentage basis but also on the basis of the absolute weights of ash per plant (figs. 6, 7, and 8). Figure 15 indicates

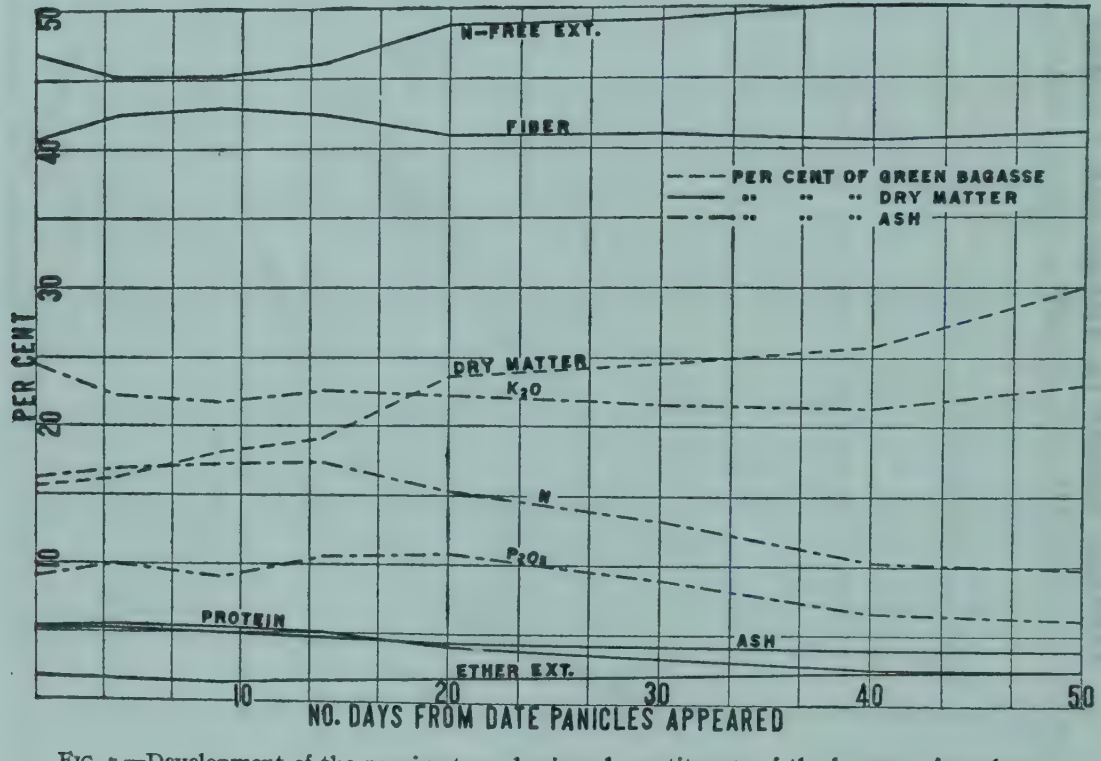

FIG. 7.—Development of the proximate and mineral constituents of the bagasse of sorghum.

that this accumulation of ash in the leaves is not due to potassium or phosphorus, for the percentage of these undergoes a marked decrease. No doubt calcium and silicon would be found responsible for the increase in mineral matter if analyses had been made for these elements. The mineral matter in the other parts of the plant remains practically constant throughout the periods of growth studied here. Figure 15 shows that potassium is more abundant than phosphorus in all parts of the plant except the tops, where the phosphorus towards maturity accumulates in greater amount. This relation is perfectly normal; it obtains in the seed of practically all plants. The prominence of nitrogen in the tops and in the leaves, and of potassium in the stalks (bagasse), is also characteristic of most plants.

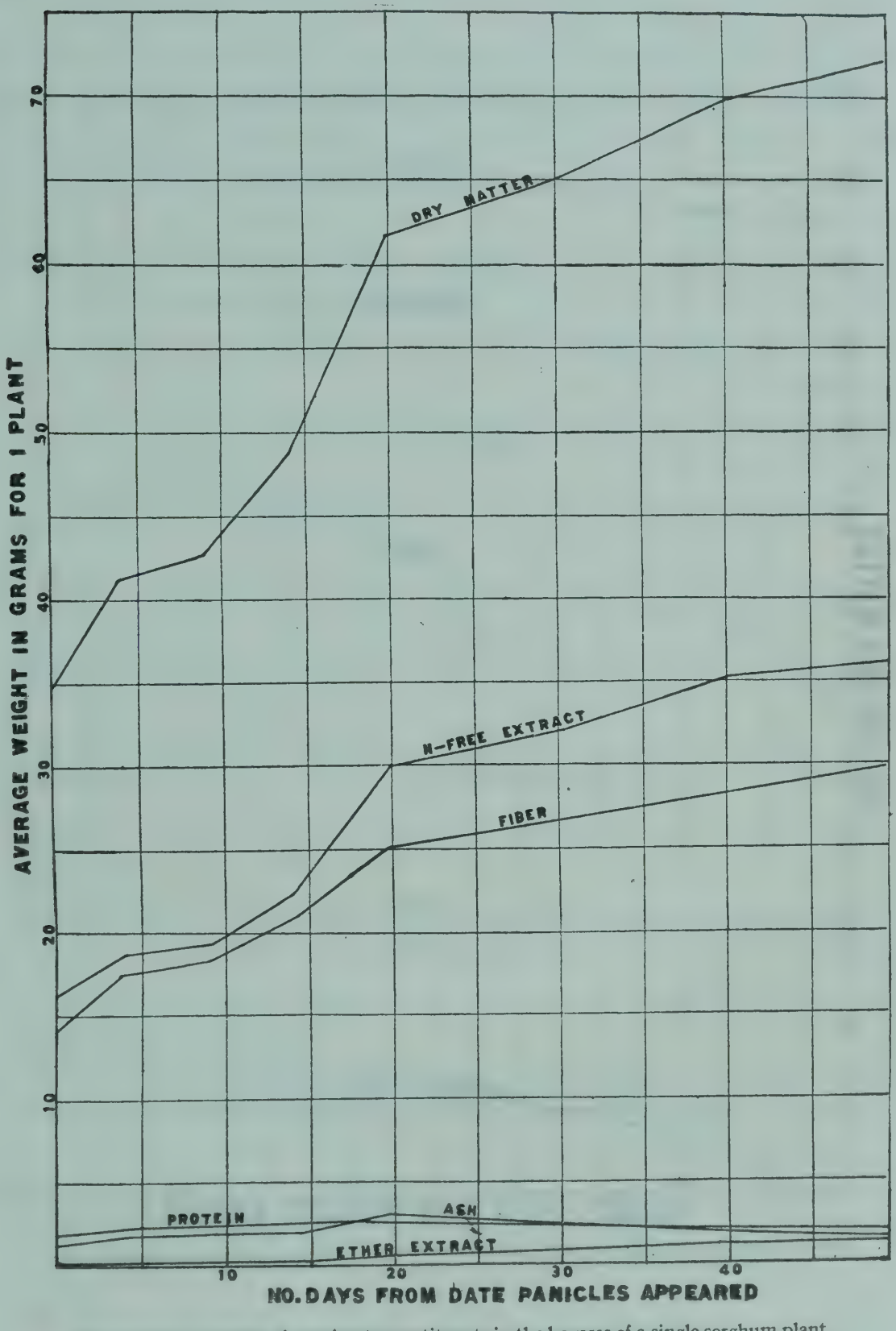

FIG. 8.—Total weight of proximate constituents in the bagasse of a single sorghum plant.

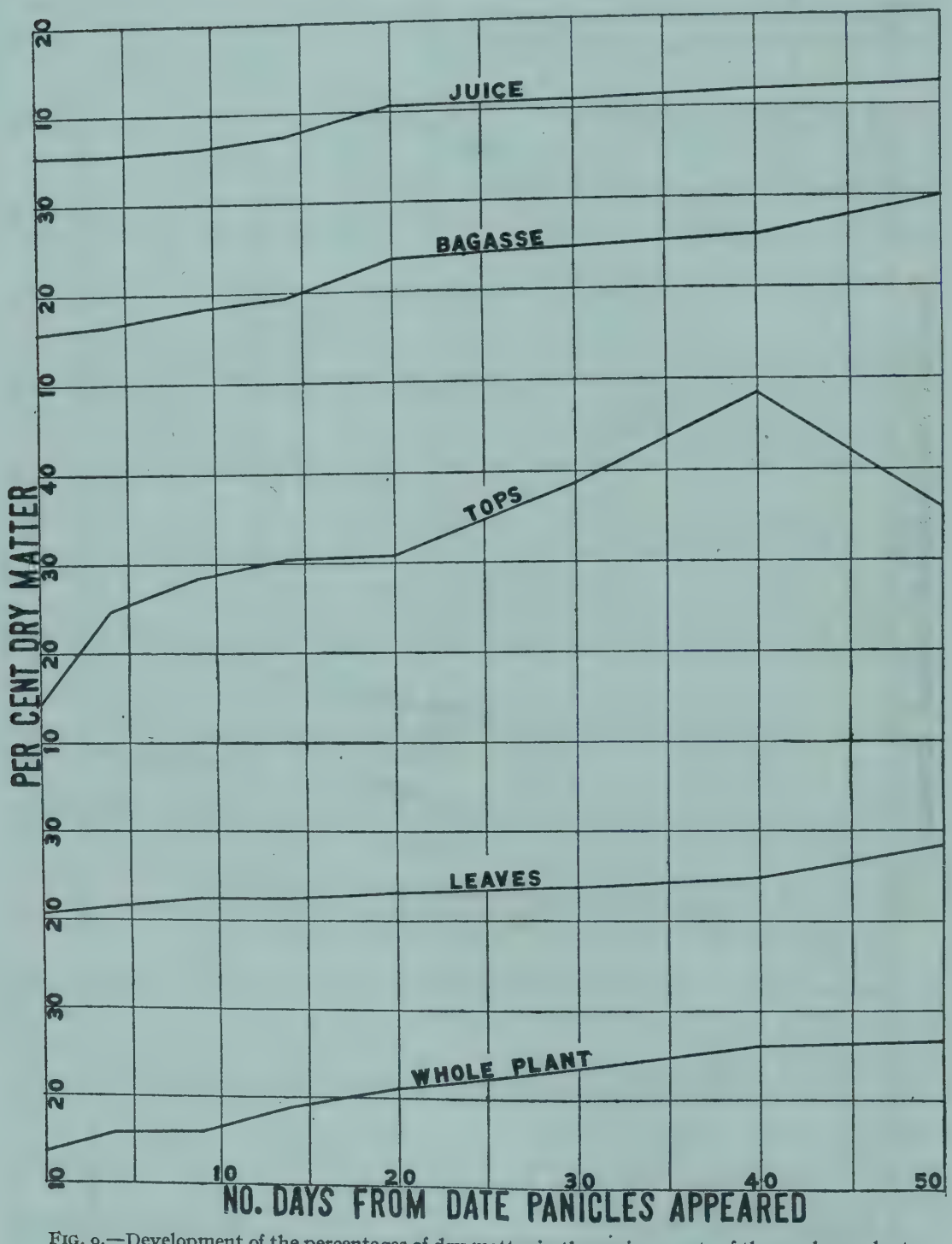

FIG. 9.—Development of the percentages of dry matter in the various parts of the sorghum plant.

V.—COMPOSITION OF THE JUICE

The juice of sorghum has naturally received greater attention at the hands of analysts than any other portion of the plant. Since, however, in practically all the previous work on sorghum only sucrose, reducing sugars, and solids-not-sugar were determined, it was thought desirable to make a more thorough investigation and attempt to acquire information concerning (1) the kinds of carbohydrates present, (2) the character of the

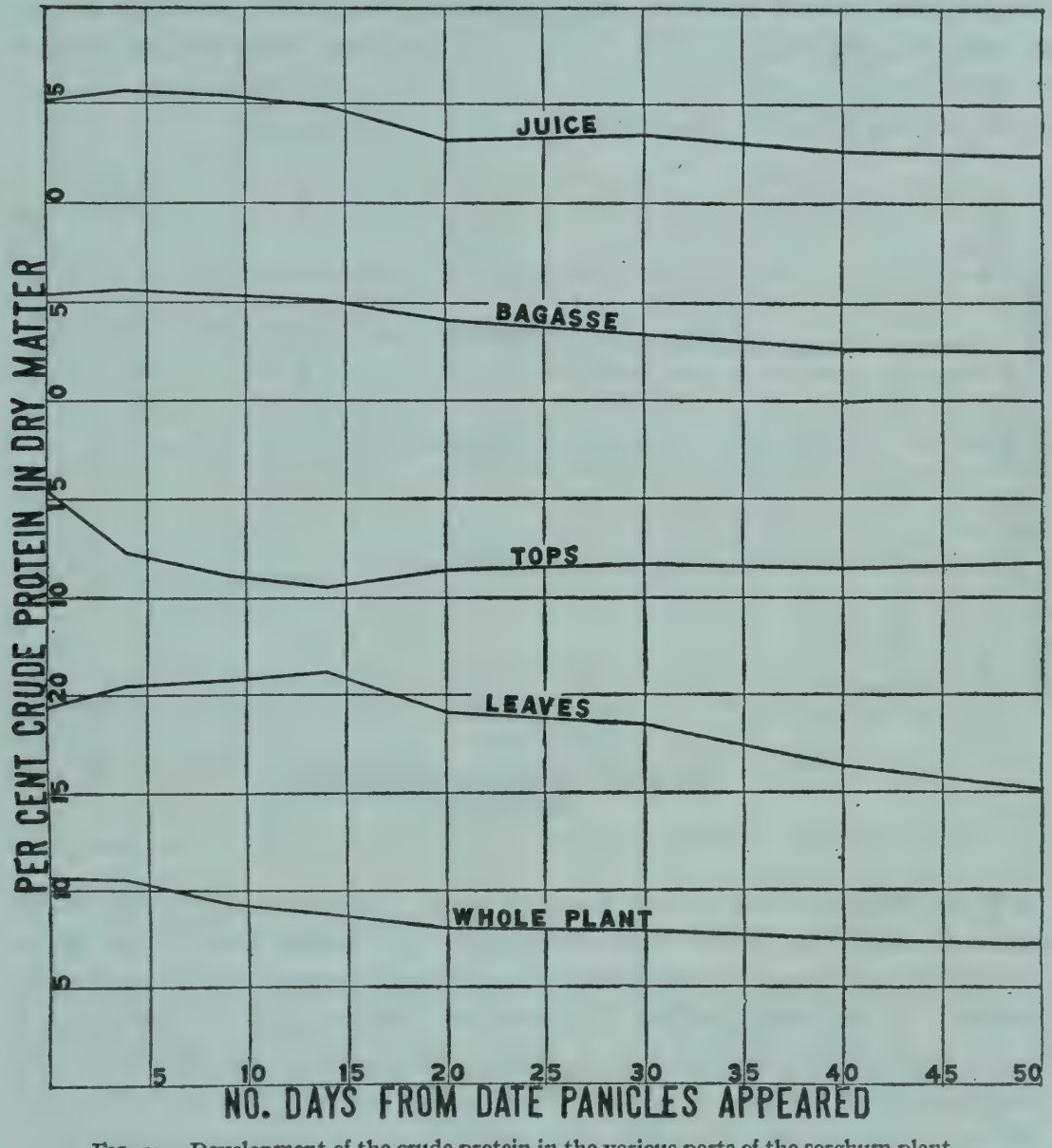

FIG. 10.—Development of the crude protein in the various parts of the sorghum plant.

noncarbohydrate solids, (3) the distribution of sugars in the cane, (4) the history of these various constituents during the growth of the plant, and (5) the effect of the removal of the seed heads on the sugar content of the juice.

(1) KINDS OF CARBOHYDRATES PRESENT.—Dextrose, levulose, and sucrose are the only sugars definitely identified. Raffinose could not be detected by the mucic acid reaction for galactose, nor maltose by the

osazone reaction. Sugar cane has also failed to yield these two sugars. Starch and gums are known to be present; but the latter have not been identified heretofore, although they are held responsible for the failure of sorghum as a source of crystallized sugar. In the sugar cane, Maxwell (*11*) found that the "so-called gums" consist largely of pentosans and hexosans. The pentosans are considered to be mostly xylan.

In the investigations reported here, the ordinary quantitative determinations of sucrose, dextrose, and levulose were also taken as means of identification when maltose and raffinose had been proved absent. Sucrose was determined by the Clerget method, and dextrose and levulose by the formula given by Wiley (*21, p. 360*). The discussion of these sugars will be given in subsections 3 and 4 of this section.

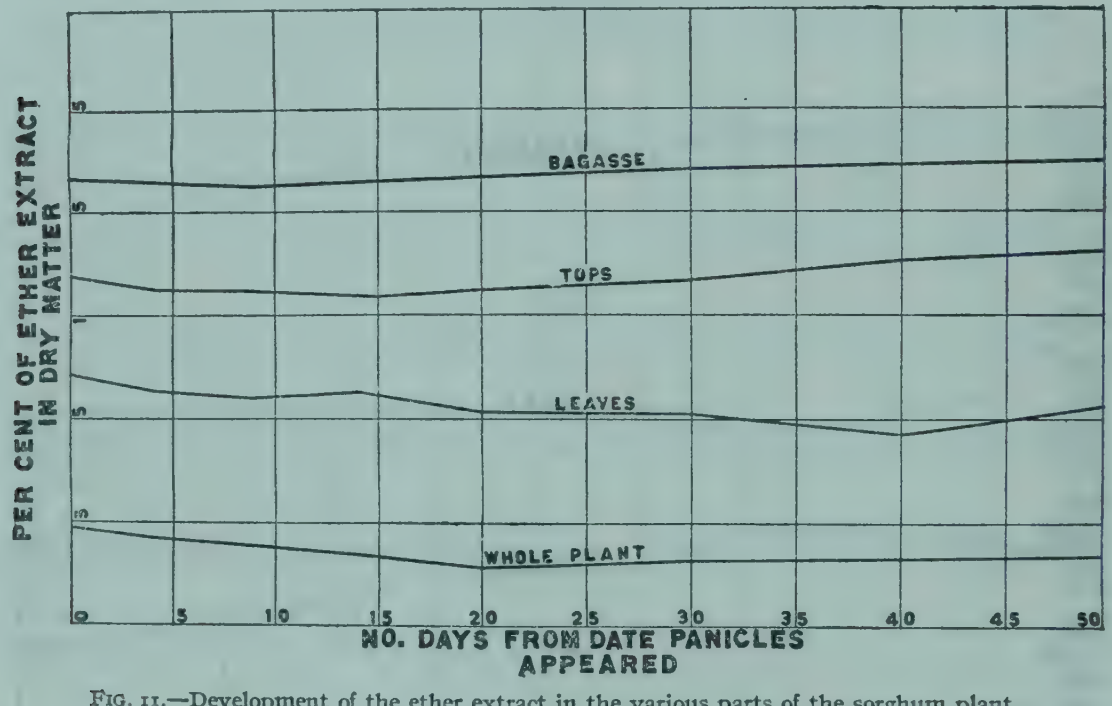

FIG. 11.—Development of the ether extract in the various parts of the sorghum plant.

The investigation of the gums and the nonsugar constituents of the juice was made the subject of a thesis by one of the writers, G. E. Holm. A barrel of juice was obtained from a sorghum factory and preserved by freezing. To a certain degree the juice was concentrated by the freezing, so that the lower portions had a slightly higher specific gravity. This was taken into account, however, in the subsequent analyses. To prove that the freezing did not alter the amount of material precipitated by an equal volume of 95 per cent alcohol, 1,000 cc. of juice were divided into two parts. One part was frozen, then thawed, and then both portions precipitated with alcohol. The unfrozen portion yielded 1.4180 gm. dry precipitate, the frozen portion 1.3940 gm.

That portion of the alcoholic precipitate which would not redissolve in boiling water was subjected to hydrolysis with dilute sulphuric acid for 20 hours. It was cooled and filtered, and then the filtrate subjected to

the tests described above for galactose, arabinose, and xylose, with the following results:

Test for xylose	+
Mucic acid for galactose	−
Osazones for galactose	−
Osazones for arabinose	−

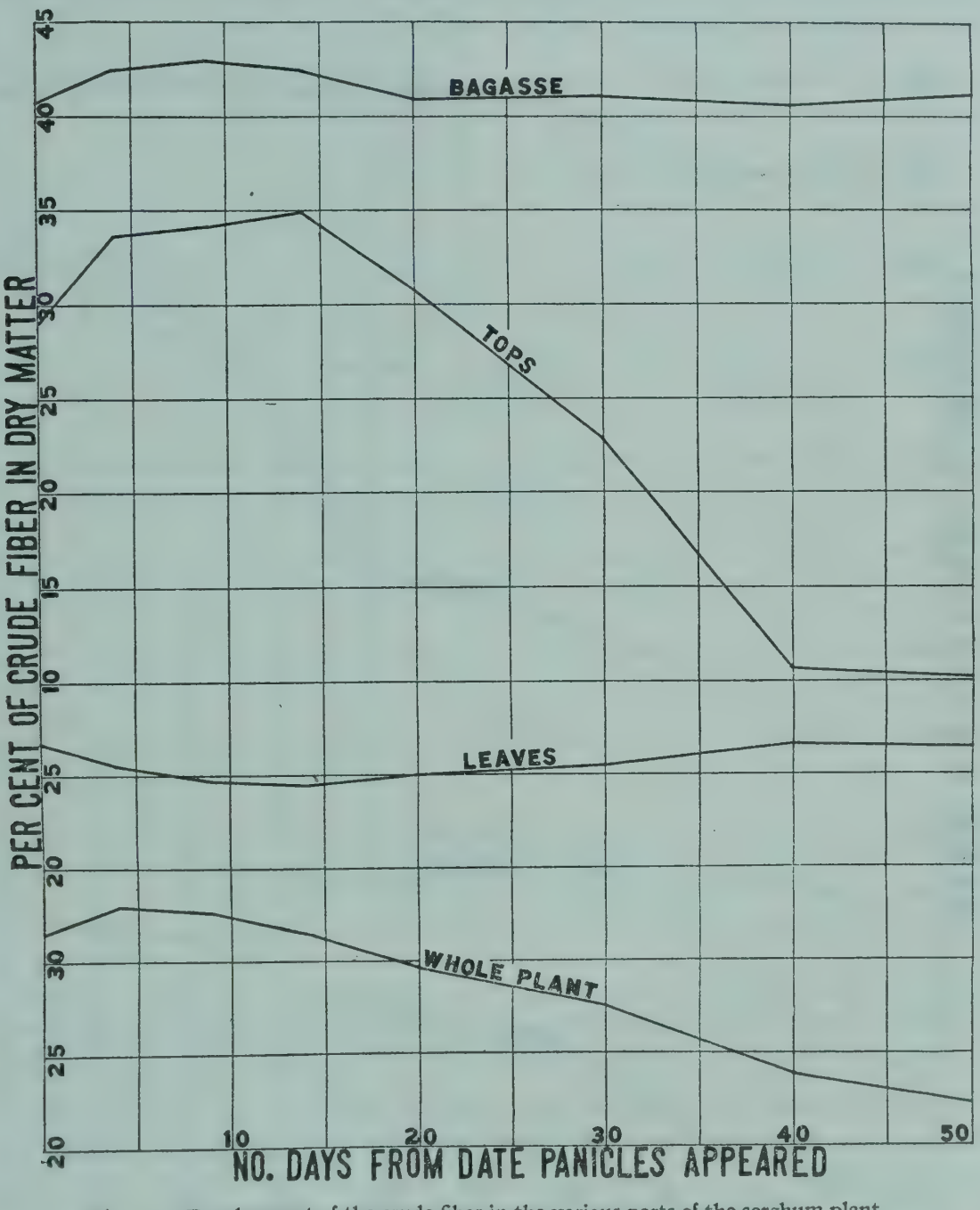

FIG. 12.—Development of the crude fiber in the various parts of the sorghum plant.

This indicates that the carbohydrate contained in the insoluble portion of the alcoholic precipitate is xylan, or at least that xylose-bearing cellular material is included in it.

The conclusion can be drawn from the above results that the material precipitated by equal volumes of alcohol from sorghum juice consists of protein, cellular material that was in suspension in the juice, and gums.

Starch is always found in sorghum juice. It will be discussed in subsection 4 of this section.

(2) THE NONSUGAR SOLIDS.—This classification will include both nonnitrogenous and nitrogenous compounds. In the former group the organic acids are the most prominent. Malic acid is usually considered

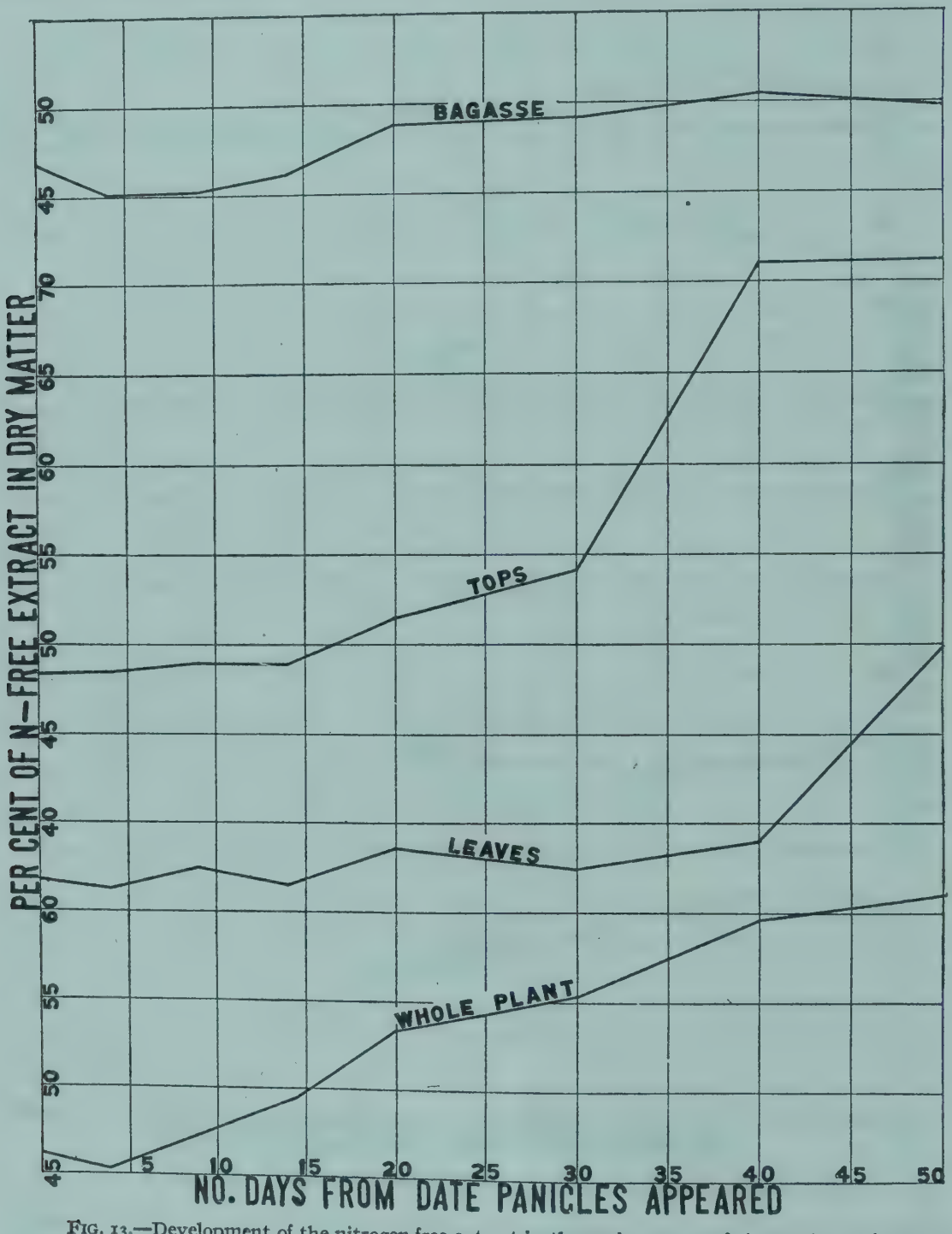

FIG. 13.—Development of the nitrogen-free extract in the various parts of the sorghum plant.

to be the characteristic acid of sorghum juice. Parsons (*12*), however, found the scale on the sugar pans to be about two-thirds calcium hydrogen aconitate. In a series of unpublished experiments by the writers, malic, citric, and tartaric acids were found to be invariably present. Also

during the isolation of nitrogenous compounds described below, calcium oxalate crystals were separated and identified in appreciable quantities. Lack of opportunity has prevented the study of the history of these acids during the development of the plant. Titration data on juices are not included in this paper. They bear little significance, since in all plant juices the acids occur as salts to a considerable degree and the titration gives no idea of the absolute quantity of acids present. Suffice it to say at this place that malic, tartaric, oxalic, citric, and aconitic acids are present in sorghum juice.

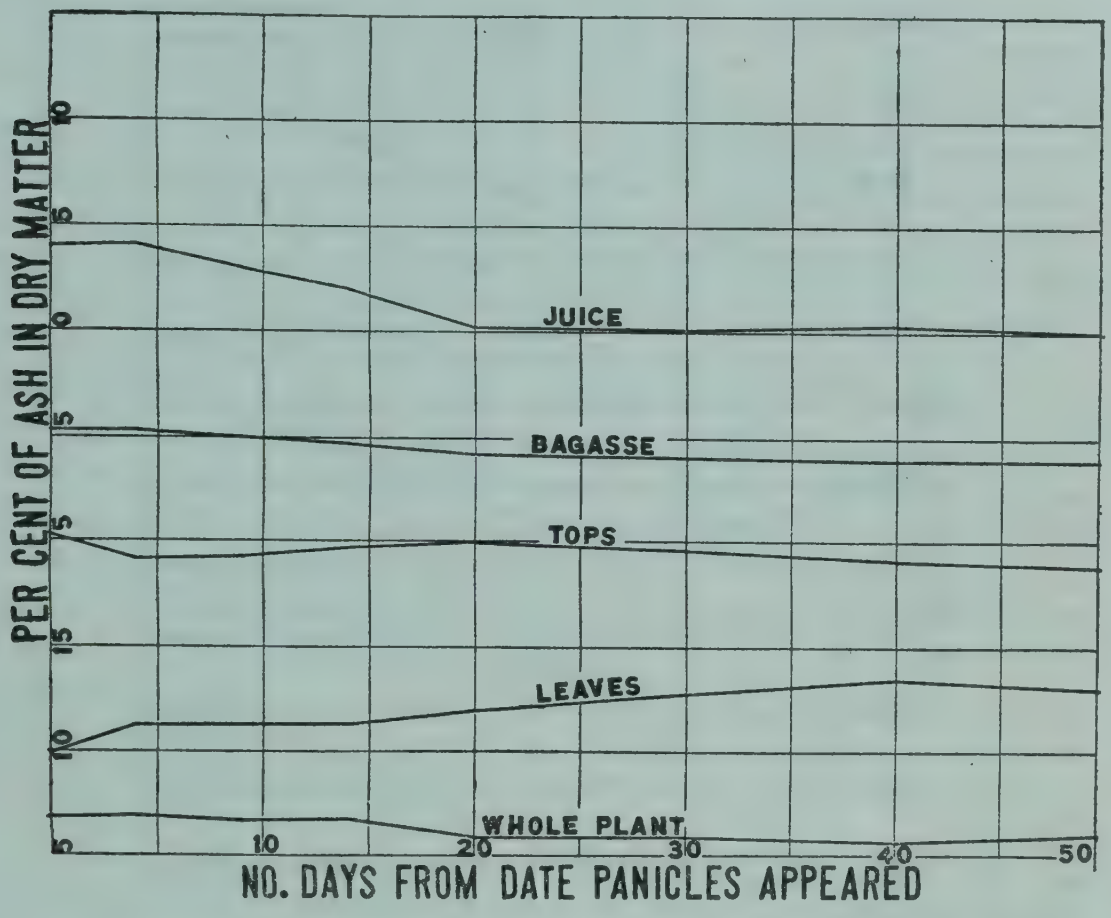

FIG. 14.—Development of the ash content in the various parts of the sorghum plant.

These data prove conclusively the presence of pentosans in the gums of sorghum juice. It is believed that the substances precipitated by alcohol are true gums and not pectins, since the jell test described by Goldthwaite (*6*) gave negative results. The ash, by qualitative tests, was shown to consist mostly of calcium and magnesium with some potassium. This is in accordance with the description of true gums by Haas and Hill (*7, p. 120*). It is also in accordance with the findings of Anderson (*3*) that sorghum juice, because of the gums present, absorbs over twice as much calcium hydrate as is accounted for by titration.

One-half liter of juice of specific gravity 1.078 was precipitated by alcohol. The precipitate was dried and weighed. A portion of it was used for ash and for nitrogen determinations. The rest was boiled in

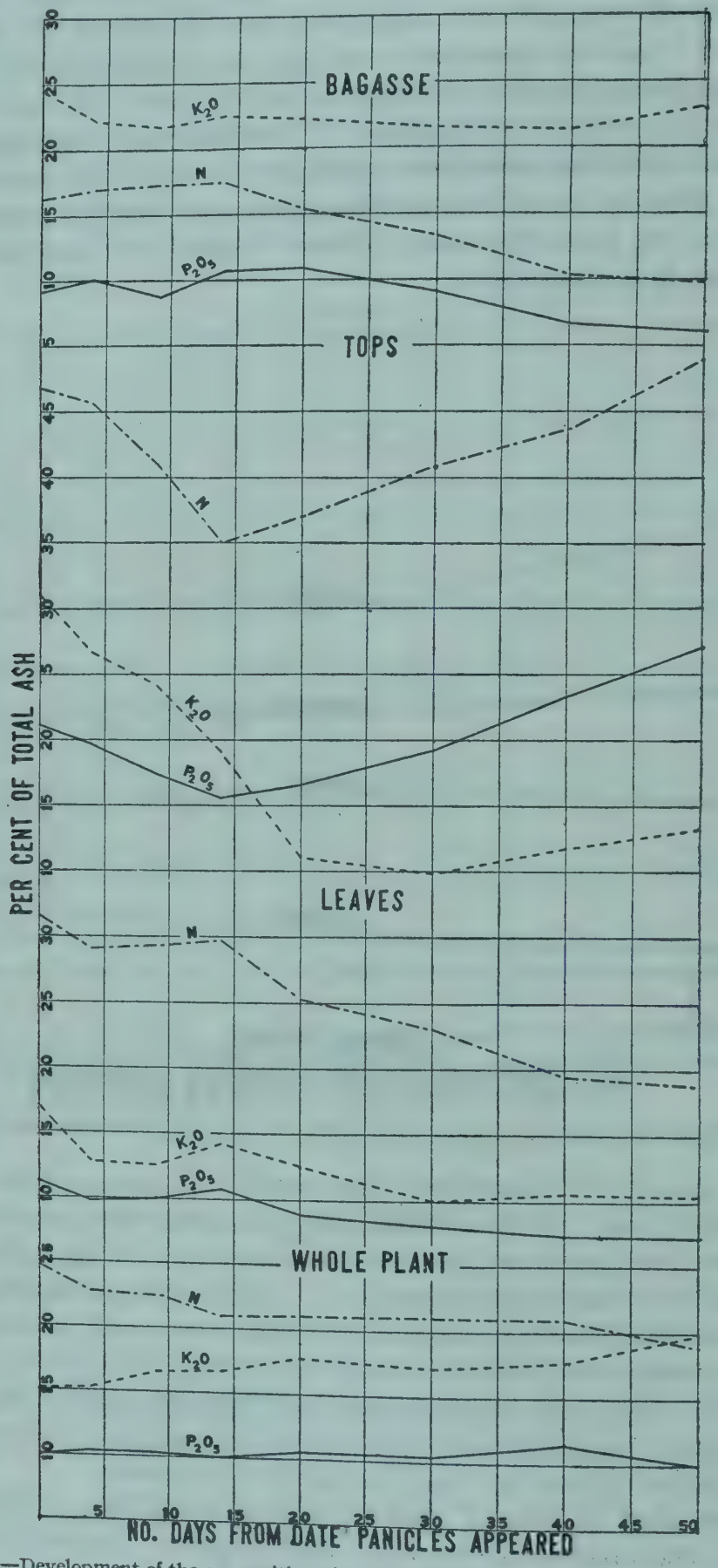

FIG. 15.—Development of the composition of the ash in various parts of the sorghum plant.

water for 15 minutes, filtered from the insoluble portion, and the soluble portion precipitated by alcohol. The results were as follows:

Total alcohol precipitate from 500 cc. juice	4.30 gm.
Percentage of juice	0.399.
Composition of precipitate:	
Proteins (N×6.25)	12.00 per cent.
Ash	22.22 per cent.
Gums (by difference)	65.28 per cent.
Solubility of precipitate:	
Insoluble in boiling water	23.87 per cent.
Soluble in boiling water, precipitated by alcohol	26.40 per cent.
Soluble in boiling water, not precipitated by alcohol	49.73 per cent.

No doubt most of the portion that does not redissolve in hot water consists of protein that was coagulated by the alcohol and of fine particles of pith and fiber that were suspended in the original juice. The high ash content is probably due to the bases that are always associated with the true gums. There is a possibility that calcium citrate constitutes a portion of it, since this salt is rather insoluble in alcohol.

That portion of the alcoholic precipitate which redissolved in water was hydrolyzed for 15 hours with *N*/5 sulphuric acid. The acid was removed with barium hydrate and the filtrate tested for xylose, arabinose, and galactose. The osazones were formed and compared with those from the pure sugars. The mucic acid test was employed for galactose, and the Bertrand reaction (*1*, *p*. *101*) for xylose. The results are as follows:

Bertrand test for xylose	−
Mucic acid test for galactose	+
Osazone for galactose	+
Osazone for arabinose	−

These results indicate the presence of galactan in the gums. It was surprising to find no pentoses in this solution, since they should be present whether the alcoholic precipitate consists of true gums or pectic substances. In the belief that the failure to find pentoses was due to the osazone method of detection and not to their absence from the solution, they were sought for by the phloroglucid method. No more sorghum juice being available, some sorghum sirup was diluted with water, the gums precipitated and reprecipitated with alcohol, dried, weighed, and analyzed, with the following results:

Ash	19.09 per cent.
Pentosan	3.76 per cent.

The nitrogenous constituents were studied in the juice preserved by freezing. The writers know of no previous work on this subject. Some rather careful work has been done with sugar-cane juice, and that is the only basis for comparison in the present instance.

Since Browne (*4*) and Maxwell (*11*) have recommended and used the ratio of protein to nonprotein nitrogen in cane juice as an indication of the stage of maturity of the plant, these determinations were made on sorghum juice by means of Stutzer's reagent (*20, p. 38*). The results for 50-cc. samples follow:

	Percentage of nitrogen in juice.	Percentage of total nitrogen as—	
		Albuminoid nitrogen.	Amid nitrogen.
Sample 1	0.0204	39.2	60.8
Sample 2	.0191	35.8	64.2

In sugar-cane juices the albuminoid nitrogen may vary from 20 to 70 per cent of the total nitrogen, depending upon the age of the juice and the method of extracting the juice. The high proportion of "amid nitrogen" in sorghum is significant from the viewpoint of sirup manufacture since it represents the impurities which are not coagulated by heat and which are not in large part removed by lime defecation. They no doubt contribute to the flavor of the sirup.

It was found that lead acetate, lead subacetate, and mercuric nitrate would each precipitate a different amount of material from the juice. Therefore these reagents were used to remove fractionally the nonsugar solids from the juice. The mercuric nitrate was added to the filtrate from the lead acetate precipitate and the lead subacetate brought down the third fraction. The following are the data for a typical example of this precipitation on a juice containing 0.0265 per cent of nitrogen:

Precipitant.	Percentage of nitrogen in juice.	Percentage of nitrogen precipitated by reagents.
Lead acetate	0.0071	27.30
Mercuric nitrate	.0063	24.23
Lead subacetate	.0062	23.84
Last filtrate	.0065	24.52
Total	.0261	99.89

Thus, three-fourths of the nitrogen can be removed by these precipitants.

Forty-eight liters of juice were treated in this way, the precipitates being separated and washed by centrifuging. The precipitates were decomposed with hydrogen sulphid, the metallic sulphid filtered off, and the filtrate concentrated *in vacuo* at 30° C. to a thin sirup. From all

three fractions brilliant octahedral crystals of calcium oxalate separated. Their crystal form, together with their solubilities,[1] established their identity as calcium oxalate. They were removed by filtration and washing and the filtrates concentrated to thick sirups. All three again produced the same kind of crystals; in this case they were spherical masses, consisting of brilliant radiating needles. The sirups were diluted with water, and the crystals filtered off and recrystallized three times. On ignition they left no ash; they dissolved in large quantities of boiling water; they decomposed at 286°–288° C. It is believed they are crystals of the "impure leucine" described by Hawk (*8, p. 492*) and by Abderhalden (*2, p. 559*), who designates them l–leucin, and states a decomposition point of 293°–295° C. Shorey (*15*) isolated a compound from Hawaiian cane juice that is no doubt identical with the present one.

The three filtrates were again concentrated to a thick sirup. That from the lead acetate fraction yielded a very small quantity of crystals of two forms—one, long prisms; the other, flat hexagons. Both were insoluble in cold water, alcohol, and dilute acetic acid, but soluble in dilute sodium-hydroxid solution. The hexagonal plates when dissolved in hot water and treated with lead acetate slowly produced a dark color, showing the possibilities of its being cystin. The amount of material available was too small to perform any further tests. The long prism-shaped crystals could not be identified, although they had the appearance of aspartic acid.

The filtrates from the last crop of crystals of the mercuric nitrate and the lead subacetate fractions, after standing for some time, deposited small quantities of wedge-shaped crystals. They were slightly soluble in water, insoluble in alcohol and in ether, decomposed at 207°–210° C. (Abderhalden states 213° C. for d–l–asparagin), were acid in reaction, liberated ammonia when treated with alkali, and gave the pyrrol test (*13*) for asparagin. These reactions indicate that the crystals were d–l–asparagin. Maxwell found both asparagin and aspartic acid in cane juices. Later, Shorey (*14*) found that glycocoll is the "principal amid" of sugar cane, and that asparagin is not present. Since our preparation liberated ammonia with alkalies, it is no doubt not glycocoll. Attempts to isolate the latter have failed.

The filtrates were neutralized with calcium carbonate, filtered, and concentrated. Nothing deposited from the lead subacetate fraction, but from the mercuric nitrate fraction needle-like crystals, interspersed with further crystals of asparagin, were found. The quantity was too small for identification, although the crystals answer the description of the glutamin identified by Zerban (*22*) in sugar-cane juice, and glutamin almost invariably accompanies asparagin in plant juices.

[1] These crystals were soluble in 5 per cent hydrochoric acid, insoluble in dilute ammonium hydroxid and dilute acetic acid.

To sum up the work on identification of compounds in sorghum juice the following list is given:

Sugars:
- Sucrose.
- Dextrose.
- Levulose.

Organic acids:
- Aconitic.
- Citric.
- Malic.
- Tartaric.
- Oxalic.

Polysaccharides:
- Starch.
- Galactans (in gums).
- Pentosans (in gums).
- Xylose (in cellulose of pith).

Nitrogenous compounds:
- Protein.
- l-Leucin.
- d-l-Asparagin.
- Glutamin.
- Cystin (?).
- Aspartic acid (?).

(3) DISTRIBUTION OF SUGARS.—From the practical standpoint, it is of importance to know the relative concentration of sugars in the various joints of the cane, since it may be unprofitable to mill the whole stalk; and from the standpoint of the physiology of the plant it is of interest to know how the concentration of sugars indicates the relative maturity of the various joints.

It is important to know also the sugar content of the leaves and of the suckers. It has been conclusively shown by many investigators (*5, p. 142; 19, p. 65*) that there is considerable sugar in the leaves but that the purity of the juice (percentage of the total solids as sugars) is so low that its sirup-making qualities are much inferior to those of the juice of the cane. Since most cane is milled either with the leaves removed or when the leaves are partially dried and hence contain but little extractable juice, the question of the leaf juice is of little importance and will not be dealt with further. The question of the juice of suckers, however, is of more importance, since under some conditions sorghum suckers badly; and when a corn binder is used for harvesting, the sucker canes are included. Here again many analyses are on record which show consistently that the suckers have a composition very similar to that of the main canes at the same stage of maturity. Since the suckers are always several stages behind the main canes in development, and since the maturity of a plot is judged by the seed heads of the main canes, the effect on the juice of cutting the two at the same

time is apparent. Collier (*5, p. 137*) says, "The suckering then of the crop, or at least the careful exclusion of suckers from that portion of the cane which is intended to be worked for sugar, is of the most imperative importance. For sugar production they are far worse than worthless. But they may be used for the manufacture of syrup, since both glucose and sucrose enter into its composition; and, in fact, the presence of the suckers in the crop would very easily prevent the crystallization of the syrup which the manufacturers of syrup frequently find a serious disadvantage." Since the analyses of suckers at this station contribute nothing new to the above facts, they will not be given here.

Collier (*5, p. 225–237*) determined the amount of sugars in the top and bottom halves of the cane and found little difference between them. In other experiments he divided the cane into thirds and again found little or no difference in the sugar content. So far as is known by the writers, no one has analyzed each joint separately. Reasoning by analogy to the suckers, the relative immaturity of the upper joints and the relative old age of the lower would lead one to expect a greater concentration of sucrose in the middle joints and of reducing sugars in the top and bottom joints.

The individual joints of several samples of cane in the dry dough stage were analyzed, with the results shown in figure 16. The concentration of sucrose and of reducing sugars varies inversely; but the variation is not proportional, since the total sugars are far higher in the middle portions of the cane. In fact, one of the most significant curves in the chart is that for the total sugars extractable per 1,000 parts of cane. This varies from 21 in the top joint to 46 in the middle and 25 in the bottom.[1] From the standpoint of sirup making, the top joint, and perhaps the bottom, could well be excluded from the milling, since they contain not only a small amount of sugar but a large amount of nonsugar solids (see the top curve in the graph), which are detrimental to good sirup making. Calculation shows that about 5 per cent of the total sugar would be lost by this practice, but this would be offset by the improvement in quality of the sirup. Went (*17*) finds about the same distribution of sugars in sugar cane as is reported above, except that there is a continuous increase in sucrose up to the joint next to the bottom.

In most plant juices the levulose exceeds the dextrose in amount, which fact is usually explained on the ground that the dextrose is more easily utilized in respiration. In sorghum juice the dextrose is always in excess of the levulose. A small portion of this dextrose may represent starch that is not yet polymerized (see next subsection for starch content of juices). It will be noticed that the excess of dextrose over levulose is least in the

[1] In explanation of the apparently very small amount of sugars extracted in the cane, it should be stated that this work was done with a small experimental mill which extracted an average of only 33 per cent of juice from the cane, whereas large mills obtain from 60 to 70 per cent.

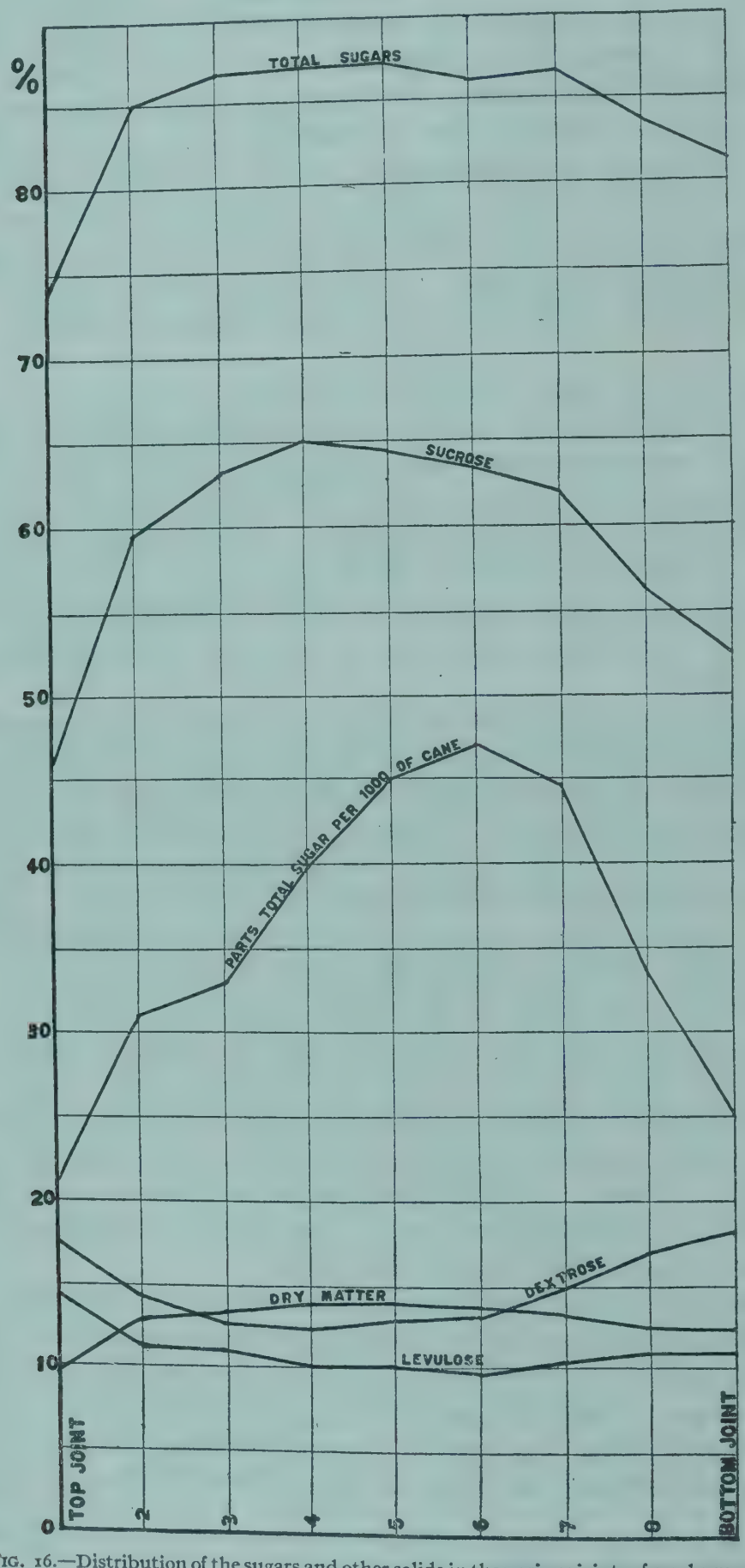

FIG. 16.—Distribution of the sugars and other solids in the various joints of sorghum cane.

upper portion of the cane, where respiration is proceeding most rapidly. This fact is in accordance with the view that the excess dextrose is the sugar most available for respiration. That portion of the dextrose which is equal to the levulose represents, of course, the invert sugar portion to be converted into sucrose.

(4) DEVELOPMENT OF THE CONSTITUENTS OF THE JUICE.—Many thousands of analyses have been made of the juice of sorghum to show the development of the various constitutents of the juice, especially the sugars. It would be fruitless to review them here, since in general they all show about the same trend of development—an increase of sucrose up to maturity, a decrease in reducing sugars, a concentration of the juice due to desiccation of the ripe cane, and a slight increase in purity (percentage of total solids as sugars).[1]

The results of the analyses at this station bring out very similar relations among the constituents of the juice. Since nothing new would be contributed by presenting all the analyses made at this station, only the work of the season of 1914 is given (fig. 17). This includes the separate determinations for levulose and dextrose, which have not been reported heretofore for sorghum juice during a whole season's development. For the sake of direct comparison, the only determinations for starch which were made, which were on the 1916 crop in connection with topping experiments, are also included in the graphs. Another fact that should be pointed out is that these analyses were made on cane grown at the northern limit of the sorghum-growing regions of the country and hence form a basis of comparison with cane grown during a longer and warmer season. To make this comparison more apparent, Collier's curves (*5, p. 169*) for the average results of analyses of Early Amber cane grown at Washington, D. C., in 1881, are also given in figure 18.

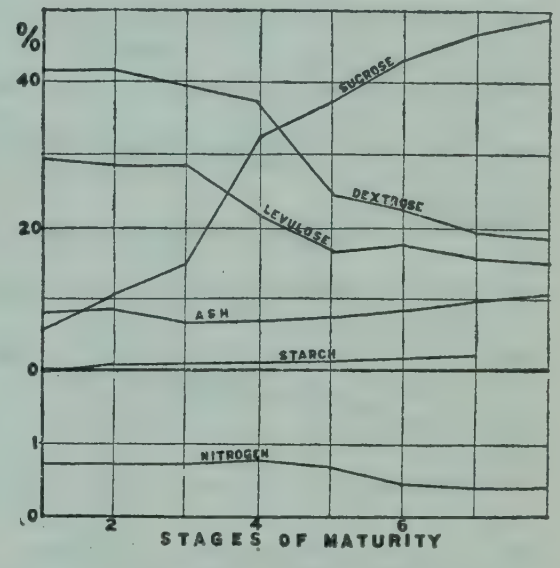

FIG. 17.—Development of the constituents of the juice of sorghum. (See p. 2 for description of the stages of maturity.)

One or two significant facts can be pointed out in the curves in figure 17. In the first place, the ratio of levulose to dextrose changes during the progress of the development of the plant. As maturity approaches, the dextrose decreases more rapidly than the levulose. At the same time, the starch increases from about 0.7 per cent to 1.8 per cent. This small amount of starch can not account for the marked decrease in dextrose.

[1] It should be pointed out that in sorghum the standard for purity is the total sugars, whereas in sugar-cane and sugar-beet work it is the sucrose alone. This is because of the fact that in sirup making the total sugars are utilized.

The latter is probably due to at least three causes: (1) Respiration, (2) conversion into sucrose, and (3) conversion to a slight extent into starch. The changes in the dextrose-levulose ratio in the juice during growth and in the various joints of the cane (fig. 16) are in the reverse order. Thus, from the standpoint of maturity, the lower joints contain the most dextrose while the older plants contain the least. No explanation is offered for this apparent anomaly.

Another significant fact brought out by the curves is the undiminished upward trend of the sucrose curve clear to the last stage analyzed. In this stage the plant was mature, judged by the condition of the seeds,

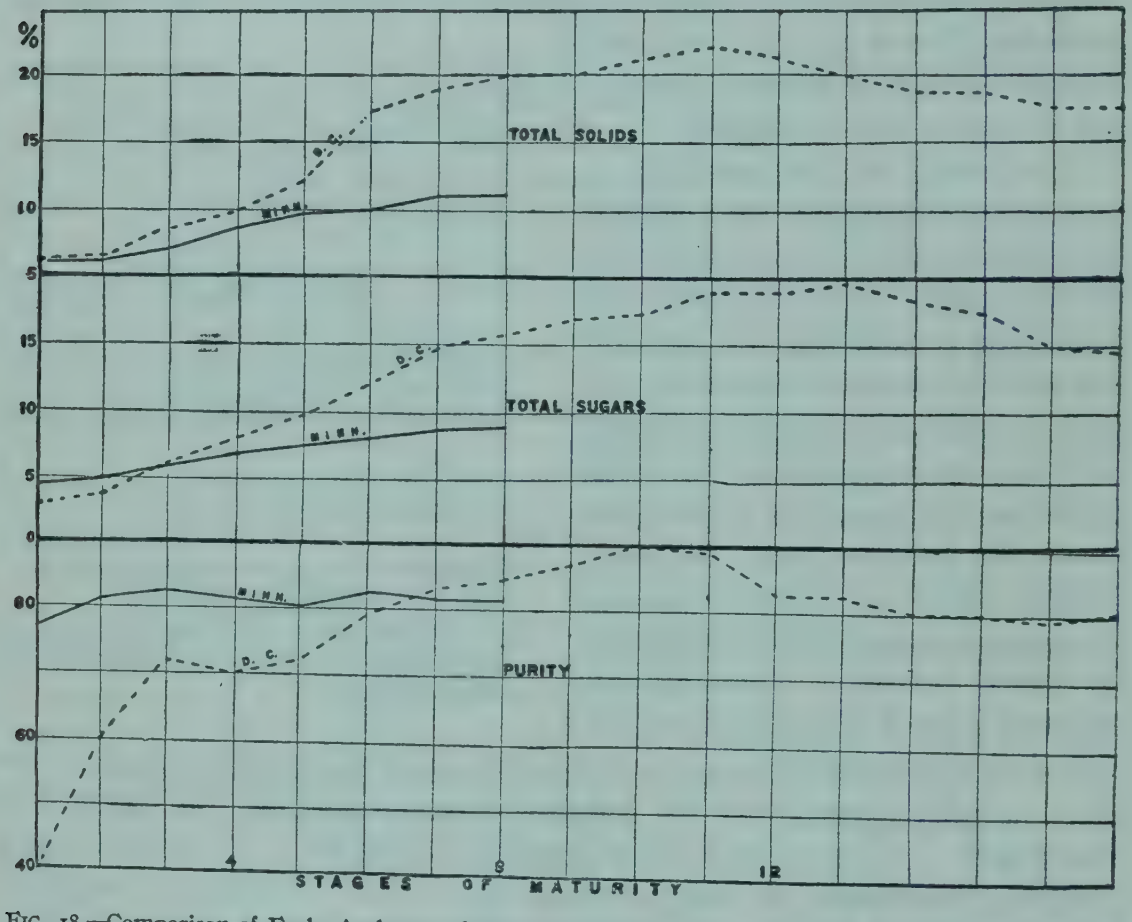

FIG. 18.—Comparison of Early Amber sorghum grown in Minnesota with that grown in the District of Columbia.

since the latter were hard and starchy and showed fair germination. It is apparent, however, that the changes in composition of the juice had not ceased. If the taking of later samples had not been prevented by frost, there is no doubt that the sucrose content would have undergone still further increases. In figure 18 there is clearly shown a continuation of increases in total sugars, dry matter, and purity in Collier's Virginia-grown cane during a period of 10 to 20 days after apparent maturity. Sorghum cane grown as far north as Minnesota probably never reaches the maximum possible content of sugars. The data in the curves are for 1914, which was a dry and hot season during the period of maturation of sorghum. Although this plant can withstand these climatic conditions

better than most crop plants, it can not develop normally, as is shown by the low content of solids in these samples and by the high purity of of the juice during the early periods. As regards the latter, it should be kept in mind that the "purity" means the relative proportion of total sugars in total solids. In an immature cane the purity is low, as is shown by the curve for the Virginia samples. The relatively high purity of the Minnesota samples is another example of the oft-observed fact that during periods of unfavorable growth conditions a plant attempts to reach maturity as quickly as possible. Usually this is evidenced in the reproductive parts alone; in the sorghum it is apparent also in the composition of the juice.

The curve representing the percentage of nitrogen in the juice remains practically level. This signifies that the absolute amount of nitrogen must increase clear to maturity. The nitrogen compounds comprise a prominent portion of the nonsugar solids, and the flatness of the nitrogen curve is in harmony with that of the purity curve.

VI.—EFFECT OF REMOVING THE SEED HEADS

There are many references in the literature on sugar-producing plants relating to the effect on the composition of the juice of removing the fruiting parts of the plant. Collier (*p. 138–140*) found that removing the seed heads at immature stages hastened the maximum production of sugar in the juice, but that this maximum was the same as that in unheaded stalks, although in the latter it was reached a week or 10 days later. Thus the effect of heading the cane was to hasten the maturity of the juice but not to increase its potential sugar content. Wiley (*19*) reports analyses which show very slight differences in favor of topping. Heckel (*9*) reports an increase in sucrose in both corn and sorghum due to removal of the floral parts. It is not clear from the latter data, however, whether only the sucrose is increased or the total sugars as well. Other work shows similar inconclusive data on this question.

For two seasons heading experiments were conducted at this station. The results are presented in Tables II and III. It will be seen that in general the data corroborate Collier's conclusions that heading merely hastens the maturity of the juice but does not affect its final composition. There is some evidence that the amount of starch produced is increased, but the differences are too small and the number of analyses too limited to warrant any definite conclusions. From the standpoint of sirup manufacture, the removal of the seed heads should be practiced in order to bring about an earlier maturing of a portion of the crop, although by so doing the value of the seed would be lost.

TABLE II.—*Effect of the removal of the seed heads on the composition of the juice*

1915 CROP

Treatment of plants.	Stage of growth when heads were removed.	Stage of growth when analyses were made.	Percentage of total solids.	Percentage of total solids as—	
				Sucrose.	Reducing sugars.
Heads off	Full bloom	Hard dough	18.0	71.7	10.1
Heads on	do	do	14.9	65.3	18.5
Heads off	Milk	Soft dough	13.0	56.9	30.0
Heads on	do	do	11.2	47.4	37.0
Heads off	Milk	Hard dough	14.3	60.8	27.5
Heads on	do	do	10.5	50.6	35.6
Heads off	Milk	Mature	15.8	64.9	18.0
Heads on	do	do	12.9	56.7	24.4
Heads off	Soft dough	Hard dough	11.6	50.9	33.0
Heads on	do	do	11.4	57.0	27.2
Heads off	Soft dough	Mature	12.8	62.8	22.0
Heads on	do	do	11.2	52.5	30.6

TABLE III.—*Effect of the removal of the seed heads on the composition of the juice*

1916 CROP

Date on which heads were removed.	Stage of growth when heads were removed.	Stage of growth when analyses were made.	Percentage of total solids.	Percentage of total solids as—		
				Sucrose.	Reducing sugars.	Starch.
Aug. 24	Panicles out	Panicles out	7.1	15.4	68.9	0.7
Sept. 7	do	Milk	13.1	41.0	38.8	2.6
18	do	Soft dough	13.6	44.3	34.0	2.1
26	do	Hard dough	14.5	63.4	25.1	.....
Aug. 24	do	Panicles out	7.1	15.4	68.9	.7
Sept. 7	do	Milk	12.4	48.3	30.9	2.8
18	do	Soft dough	13.7	42.9	39.7	1.6
26	do	Hard dough	10.7	43.8	42.7	.....
Aug. 24	Full bloom	Full bloom	7.7	27.1	58.5	1.0
30	do	Milk	10.4	36.3	45.0	1.5
Sept. 8	do	Soft dough	15.8	49.8	27.2	3.1
18	do	Hard dough	15.2	49.8	33.2	2.6
26	do	Mature	15.9	64.7	23.2	.....
Aug. 30	Milk	Milk	10.8	39.6	42.3	1.2
Sept. 7	do	Soft dough	14.2	58.5	24.4	2.8
18	do	Hard dough	14.5	63.3	23.2	2.7
26	do	Mature	17.0	41.1	41.4	.....
8	Soft dough	Soft dough	12.8	43.5	31.2	1.7
18	do	Hard dough	13.1	60.4	24.3	1.6
26	do	Mature	13.9	43.0	40.7	.....

VII.—SUMMARY

(1) Three varieties of sorghum cane were used for studying the progressive development of the plant and the chemical composition of the various parts of it.

(2) The relative proportion of leaves, seed heads, and clean cane by weight in both the fresh and the partially dried condition was determined for one season.

(3) Considering the whole plant, there was found to be a continual increase in dry matter up to maturity. The percentage of crude fiber decreases at practically the same rate as that at which the soluble carbohydrates increase. The crude fat, ash, and protein percentages remain almost constant throughout the periods of growth studied.

(4) The computation of the total quantities of each constituent present in the plant at the various stages of growth brings out the fact that this plant builds up during the earlier part of the season its cellular structure of fiber, protein, and mineral matter, and that the later stages of growth consist in the filling up of these tissues with carbohydrates (starch in the seed, sugars in the stalk).

(5) No evidence was found which would indicate that the leaves are deprived of their carbohydrates to supply the stalk, at least during the periods of growth studied. The older the plant, the higher is the feeding value of the leaves.

(6) The maturation of the seed heads consists almost entirely in the filling out of a fiber and protein framework with starch.

(7) There is a considerable accumulation of mineral matter in the leaves, due probably mostly to calcium and silicon.

(8) Large quantities of juice were employed in isolating and identifying the nonsugar solids. Equal volumes of alcohol threw down a preprecipitate which consisted of three portions: (a) proteins, (b) cellular material in suspension, arising from the crushing of the fiber in the mills, and (c) true gums.

(9) The gums are complexes of galactan and pentosans, with about 20 per cent of mineral matter, principally calcium, magnesium, and potassium.

(10) The organic acids found in sorghum juice are aconitic, malic, citric, tartaric, and oxalic.

(11) Nonprotein (amid) nitrogen is very high in sorghum juice, even in mature cane. This is an important contributing factor in the difficulties of defecating sorghum juice for either sirup or sugar production.

(12) The following nitrogenous substances were identified in sorghum juice: l-leucin, d-l-asparagin, glutamin, cystin (?), and aspartic acid(?).

(13) The juice of suckers has a composition similar to that of the main canes at the same stage of maturity. They are, however, usually from one to three weeks behind the main canes in maturity.

(14) The middle joints of the cane are higher in total sugars and in sucrose but lower in dextrose and in levulose, than the upper and the lower joints. The upper joint contains so little sugar and such a low coefficient of purity that it can well be excluded from the milling in sirup making.

(15) Sorghum cane grown in Minnesota has a much lower sugar content than cane grown in regions of longer and warmer growing seasons. There are indications that if the advent of frost could be delayed, cane which is usually considered mature would continue for another week or 10 days not only to increase the ratio of sucrose to reducing sugars but to elaborate more total sugars. The juice of northern-grown cane has a higher purity than that of southern grown. This is a phenomenon of early maturation exhibited by most plants when grown under suboptimum conditions.

(16) At the time of the first appearance of the panicles the reducing sugars are greatly in excess of the sucrose. The former rapidly decrease and the latter rapidly increases, until at the stage of full bloom they are about equal in amount. The respective changes continue up to maturity, when the ratio of sucrose to reducing sugars is in Minnesota-grown cane about 70 to 30. In very mature Virginia-grown cane the ratio is 90 to 10, or even higher.

(17) Removal of the seed heads prior to maturity hastens the production of the maximum amount of sugar in the juice, but the same maximum would be attained later without the removal of the seed heads.

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SIMPLE METHOD FOR MEASURING THE ACIDITY OF CEREAL PRODUCTS: ITS APPLICATION TO SULPHURED AND UNSULPHURED OATS[1]

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INTRODUCTION

Much attention has been given during recent years to acidity determinations in connection with the analysis and valuation of cereals and their various products. Some of the newer literature on this subject is reviewed in a recent article by Lüers and Adler (*11*).[2] Of the methods proposed by various investigators, that of Schindler (*12*) has been rather extensively used in this country (*5*, *8*, *18*), especially in work with corn (*Zea mays*). Its use is also recommended in a recent French paper by Leprince and Lecoq (*9*).

Besley and Baston (*5*) alone have investigated approximately 10,000 samples of corn by means of a modification of Schindler's method; and the acidity values thus obtained are considered by Winton and his coworkers "the best chemical means of detecting actual spoilage, or at least a tendency in that direction" (*18*, *p. 1*). The method involves extraction of the material for 16 to 18 hours at room temperature with neutral alcohol (specific gravity 0.86) and titration of the alcoholic extract with standard alkali. The acidity is stated in terms of cubic centimeters of normal alkali required to neutralize the acidity of the extract from 1,000 gm. of material. The acidity figure 30 is taken as an arbitrary limit, beyond which the value should not rise for sound corn.

This alcohol extraction method has several weak points, as will at once become apparent if we consider for a moment the nature and mode of formation of the acids present in grain extracts. Lüers and Adler (*11*) have shown conclusively that in extracting barley or malt, acid-forming ferments come into play, and that it is therefore necessary to make a distinction between the original acidity of the material and the acidity formed during extraction. According to the same authors, the acidity of barley or malt extractions is due mostly to acid phosphates, which are partly present as such and partly are formed during extraction from organic phosphorus complexes through the action of specific ferments, to which they give the name phosphatases. The

[1] An abstract of this paper was read before the thirty-third annual convention of the Association of Official Agricultural Chemists at Washington, D. C., in November, 1916.

[2] Reference is made by number (italic) to "Literature cited," p. ——.

solubility of those acid phosphates is declared to be greatly diminished in the presence of alcohol, a fact which had previously been recognized by Weiss (*17*) and by Swanson (*14, 15*). Weiss found that if ground barley is first extracted with 96 per cent alcohol a subsequent extraction with water yields a far smaller quantity of soluble phosphorus compounds than a direct water extraction. Of the total phosphoric acid in 100 gm. of barley, 79 per cent was found to be normally soluble in water by 18 hours' extraction at room temperature with addition of toluol. If the same material was first treated with 96 per cent alcohol in a Soxhlet extractor for 20 hours, a subsequent extraction with water yielded only 45 per cent of the total phosphoric acid contained in the meal, while not more than 3.66 per cent had been removed by the alcohol. Weiss seeks to explain this difference by assuming that the high phosphorus content of the water extract is due to the action of enzyms, which he thinks are destroyed by the alcohol treatment. This explanation, however, is not in harmony with the more recent findings of Adler (*1, 2, 3*). This investigator has made extensive studies of the various groups of acid-producing ferments which come into play when ground barley or malt is extracted with water. He found that these ferments, and particularly the phosphatases, are very resistant not only to the action of the common disinfectants such as chloroform, toluol, hydrogen peroxid, etc., but also to dry heat, and that their activity is not impeded by cold alcohol, even if present in high concentrations.

It does, of course, not necessarily follow that the results obtained with barley by these investigators will apply, without qualification, to corn, oats, or rye. However, there is a strong probability that similar conditions will prevail in all gramineous seeds; at any rate, the results obtained with barley in Germany deserve the full attention of everyone who is working along similar lines with other cereals. Schindler, for example, would probably have hesitated in devising his above-mentioned alcohol extraction method for determining corn acidity had he read the paper of Weiss (*17*) which was published two years previously.

While recognizing the impossibility of suppressing acid-producing ferments by means of cold alcohol, Lüers and Adler (*11*) found that a brief boiling with 96 per cent alcohol completely destroys these ferments. They therefore make this boiling with neutral 96 per cent alcohol part of their method for measuring the original acidity of ground barley and malt. During the boiling, which is done in open beakers with occasional stirring, most of the alcohol is driven off, and when the material has reached a doughy consistency it is allowed to cool. Distilled water is then added and the mixture permitted to stand for several hours with occasional stirrings and the addition of toluol. The weight of the total amount of liquid is then determined, and after the liquid is filtered a suitable aliquot is titrated with *N*/10 alkali. Lüers and Adler are well aware of

certain inaccuracies of this method. For example, they point out that by boiling the grain material with 96 per cent alcohol certain proteins are coagulated and that in being thus transformed these bodies possess the power either to absorb acids or to combine with them. Part of the grain acidity might thus be occluded and escape detection. However, these authors recommend the above procedure for want of a better means of measuring the quantities of acid originally present in barley or malt.

ACIDITY VALUES OBTAINED BY THE ICE-WATER METHOD AS COMPARED WITH THOSE OBTAINED BY THE SCHINDLER METHOD

The method proposed in this paper was first used during a study on the diastatic power of oats. In that study the ice-water extraction method recommended by Thatcher and Koch (*16*) was employed; but it was thought necessary to neutralize each oat extract before determining its diastatic strength, inasmuch as the oats in question represented different degrees of soundness and some of them had been subjected to the sulphuring process as now practiced by the trade (*13*). In neutralizing the different aqueous oat extracts with *N*/10 sodium hydroxid very pronounced differences in acidity were observed in samples which, when the above-mentioned aclohol extraction method of Schindler was used, had shown no appreciable difference in acidity. Furthermore, the values obtained by simple titration of the ice-water extracts appeared to represent the actual acidity of the respective sample far more truly than those obtained by means of the Schindler method. The data compiled in the subjoined table will serve to illustrate these relations. The second and third columns of figures denote cubic centimeters of normal alkali which were required to neutralize the acidity in the extract from 1,000 gm. of dry material.

The samples 1s to 10s in the following table represent the same oats as No. 1 to 10. The latter set had been taken before, and the former after, the sulphur bleaching. These duplicate samples of oats of the 1915 crop had been secured by official inspectors at commercial elevators in Chicago. Since most of these oat shipments when arriving at the Chicago market appeared discolored or otherwise damaged, the dealers had tried to improve their appearance by means of sulphuring.

TABLE I.—*Acidity values of freshly ground oats and of corn meals, shown by two different methods* [1]

Sample No.	Schindler's alcohol extraction method.	Ice-water extraction method.
Unsulphured oats:		
1	33.6	18.3
2	48.2	18.5
3	41.2	17.3
4	41.0	13.4
5	45.0	21.7
6	36.5	17.0
7	42.4	17.0
8	49.2	16.3
9	42.0	11.3
10	44.0	14.9
Sulphured oats:		
1S	55.0	91.8
2S	34.6	54.0
3S	46.8	46.9
4S	47.0	42.8
5S	41.6	41.5
6S	35.6	35.6
7S	28.2	28.4
8S	53.3	27.5
9S	42.6	20.0
10S	39.0	27.4
Corn meal (degerminated):		
11	13.0	11.0
12	31.6	10.1
Corn meal (whole kernel):		
13	109.6	106.0
14	154.4	59.0

[1] Expressed in cubic centimeters of normal alkali required to neutralize the extract from 1,000 gm. of material.

In making the above determinations, all samples, after being ground to the same degree of fineness, were dried over calcium chlorid in a large air-tight cabinet with an inside temperature of 38° to 40° C., the air being kept in circulation by means of an electric fan. This mode of drying at low temperature was adopted because the oat samples, as indicated above, were also tested afterwards for their diastatic strength. The ice-water extractions were carried out in a cold-storage compartment of the Bureau of Chemistry, which is kept at a temperature close to 1° C. Distilled water of the same temperature was kept on hand. A weighed quantity of dried sample, usually 16 gm., was placed in the proper extraction bottle and the latter allowed to stand in the cold-storage room until its contents had reached the desired temperature. One hundred and sixty cc. of precooled distilled water were then pipetted into each bottle, the latter closed with a tightly fitting rubber stopper and shaken vigorously at 15-minute intervals. At the expiration of the extraction period (one hour for oats) the contents of each bottle were poured upon dry folded filters (S. & S.

No. 588 Falten filter). By pouring back the first few cubic centimeters of filtrate a homogeneous extract was obtained which, as a rule, was clear and easy to filter when it was obtained from sulphured oats. The extracts from unsulphured oats filtered more slowly and gave turbid filtrates. This turbidity, however, did not interfere with the subsequent titration. Usually when the ice-water extracts of sulphured oats were neutralized a white amorphous precipitate was formed, while with unsulphured oats no such precipitation took place. With the corn meal samples 13 and 14 similar precipitations occurred upon neutralizing. It was thought that through the high acidity of these samples some constituents had been dissolved which are insoluble in neutral liquids. However, this explanation does not cover all cases, since precipitation also occurred upon neutralizing the ice-water extracts of sample 7s, 9s, and 10s in the foregoing table, as well as the extracts of unsulphured samples 63 and 74 in Table V. Since the latter samples were two years old it is possible that the age of a sample bears some relationship to the formation of this neutralization precipitate. All manipulations of the ice-water method were carried out in the cold-storage compartment with the exception of the titrations. For the latter, a suitable aliquot of the respective filtrate was pipetted out, usually 40 cc. Phenolphthalein was very satisfactory as indicator, giving a sharp endpoint. It was employed in a 0.5 per cent solution in 50 per cent alcohol, not more than five drops were added to any of the aliquots, and the appearance of a faintly reddish tint was taken as the endpoint of titration. Methyl red could not be used in this work. In determining the acidity by the alcohol extraction method the directions given by Besley and Baston (5) were followed. The endpoint of titration of the alcoholic extract is not very sharp, a fact which was recognized by Besley and Baston in their bulletin and which constitutes one of the drawbacks of their method.

From Table I it is seen that the acidity values of natural unsulphured oats obtained at commercial elevators are in every case higher if determined by Schindler's alcohol method than if determined by the simple ice-water extraction method. If the latter values are used as a basis of calculation, these differences show an average for samples 1 to 10 of well over 250 per cent, the smallest difference being 184 per cent and the largest 372 per cent. They are therefore quite appreciable and are doubtless to a large extent attributable to the alcohol content (7) of the extract made by the Schindler method. Differences of the same general kind are shown by the corn meal samples 11 to 14, all of which had been milled three years previously, samples 12 and 14 having been dried with artificial heat and samples 11 and 13 having been stored without drying.

After being bleached with sulphurous acid, oats would naturally be expected to possess a higher degree of acidity than before the bleaching.

As shown in Table I, this view was confirmed in every instance where the acidity had been determined by the ice-water method. For example, with sample 1 the acidity before bleaching was 18.3, and after bleaching 91.8, or five times as high. While less pronounced in samples 2 to 10, the rise in acidity due to sulphuring was distinctly evident in each case. With the Schindler method, as modified by Besley and Baston, the increase in acidity due to sulphuring was hardly, if at all, apparent. In fact, in 5 of the 10 cases the figure for the sulphured sample was even lower than that for the same oats before sulphuring.

When comparing the results obtained by the two methods on the sulphured samples 1s, 2s, 3s, and 7s (see Table I), we find that, contrary to the results with unsulphured samples, the alcohol extraction method gave here lower values than the ice-water method. In other words, while practically all the acid of the oat had passed into the ice-water extract in an hour's time, the 18-hour digestion with 80 per cent alcohol had not effected the solution of all acid constituents. Thus, for sample 1s the ice-water method yielded the acidity figure 91.8; and Besley and Baston's modification of the Schindler method yielded only the figure 55, a deficiency of 40 per cent. While it would not be difficult to offer an explanation for this finding [1] I will confine myself here to a comparison of the values obtained by the two methods.

From the above experimental results one can not escape the impression that there are a number of objections to the use in work of this kind of the Schindler method as modified by Besley and Baston. Briefly summarized, these objections are:

(1) The lack of a definite endpoint of titration.

(2) The misleading character of the results obtained by the method, inasmuch as:

(*a*) The acidity values of raw, unsulphured cereals are invariably too high.

(*b*) The acidity values for sulphured cereals (oats) are frequently too low.

(*c*) The increase in acidity which always occurs when grain is sulphured frequently fails to find expression in the results, the latter quite often indicating instead of the actual increase an apparent decrease in acidity upon sulphuring, due to incomplete extraction of the acid.

The above statements refer to results obtained with freshly ground material. It may be mentioned also that for the grain dealer or manufacturer, as well as for the commercial chemist, the high price of pure alcohol would make this method rather expensive. The cost factor would, of course, be of only secondary importance, provided that the

[1] I may only point to the close similarity between this result and the findings of L. Weiss (17), which are discussed in the introduction of this paper.

method furnished as accurate a means for measuring grain acidity as has been claimed by its users. Unfortunately, my findings in the above Table I in conjunction with those recorded in the previous paper (7) point to the contrary conclusion.[1]

While for work on freshly ground sulphured or unsulphured oats the Schindler method is clearly inadequate, yet the figures obtained for corn by Besley and Baston (*6*) would seem to bear a certain relation to the relative age or soundness of the respective samples. But the changes which they have measured can not represent true acidity only. They are doubtless due, in a large measure, to the formation of amphoteric protein cleavage products which cause the titration value to increase suddenly in the presence of alcohol to several times its former magnitude.[2]

It was thought advisable that another chemist should examine a number of cereal products for acidity by both the proposed ice-water method and the Schindler method as modified by Besley and Baston. Mr. M. G. Mastin of the Bureau of Chemistry kindly undertook this task, and his findings are given in Table II.

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[1] In the latest modification of their method Besley and Baston (*6*) apply an electric mixing apparatus by which they find it possible to reduce the time of extraction in their alcohol method to 30 minutes, as against 16 to 18 hours, the time prescribed in their former paper. The introduction of this electrical device, which by the way must be rather expensive, is merely an improvement in the technic. Had the authors at the same time abolished the use of alcohol, a real improvement of their method would have been attained. Since in the 18-hour extraction with alcohol the latter had been prescribed for the purpose of preventing bacterial or enzymic action, there seems to be no longer any reason for its use, if the time of extraction is reduced to 30 minutes. From the data given in this and the preceding article, it is evident that the errors introduced by the use of alcohol must far outweigh its possible usefulness in a 30-minute extraction.

[2] One might be tempted to believe that, since by using Besley and Baston's method the acidity values of freshly ground, untreated cereals appear to be always higher than the values obtained by extraction with ice water, it should be possible to calculate the ice-water acidity from the figures published in Besley and Baston's paper (*6*). However, this would not be permissible, since the higher values obtained by their method depend not alone on the concentration of the alcohol, which is the same in each case, but also on the nature and concentration of those amphoteric grain constituents which are responsible for the shift in acidity in the presence of alcohol. The concentration of these bodies naturally varies from case to case.

TABLE II.—*Further comparisons between the ice-water and the alcohol method, showing increase in acidity which takes place in ground cereals upon standing*[1]

[Determinations made by Mr. M. G. Mastin]

Sample No.	Crop year.	Schindler's alcohol extraction method.	Ice-water extraction method.	Remarks.
Unsulphured oats:				
15	1916	42.9	16.7	Freshly ground.
16	1915	27.5	19.8	Do.
17	1916	39.0	13.7	Do.
18	1915	30.0	13.8	Do.
18r	1915	.....	31.0	After standing.
19	1916	39.0	16.7	Freshly ground.
20	1915	32.0	17.0	Do.
21	1915	27.2	14.0	Do.
21r	1915	104.0	24.0	After standing.
22	1915	15.4	14.5	Freshly ground.
22r	1915	129.0	37.5	After standing.
23	1914	13.4	8.5	Freshly ground.
23r	1914	57.0	20.2	After standing.
23rr	1914	149.0	43.0	After long standing, moldy.
24	1914	13.6	11.5	Freshly ground.
24r	1914	82.2	21.5	After standing.
25	1914	13.6	13.0	Freshly ground.
25r	1914	56.6	22.5	After standing.
26	1914	20.6	15.0	Freshly ground.
26r	1914	121.0	46.0	After standing, moldy.
27r	1914	88.8	35.0	After standing.
27rr	1914	173.5	38.6	After long standing, moldy.
28r	1914	136.6	36.0	After standing.
29r	1914	47.6	19.0	Do.
30r	1915	63.0	20.0	Do.
31	1916	36.0	22.0	Freshly ground.
32	1916	26.6	12.0	Do.
33	1916	26.6	16.0	Do.
34	1916	36.6	17.0	Do.
35	1916	35.9	18.0	Do.
36	1916	36.0	16.0	Do.
37	1916	41.5	16.5	Do.
38	1916	53.7	17.3	Do.
39	1916	60.5	16.0	Do.
40	1916	52.5	14.7	Do.
41	1916	26.0	16.0	Do.
42	1915	34.2	19.3	Do.
Sulphured oats:				
15s	1916	48.7	21.0	Do.
16s	1915	37.2	40.3	Do.
17s	1916	35.0	32.7	Do.
18s	1915	28.0	22.5	Do.
18sr	1015	126.0	21.6	After standing.
19s	1916	29.0	33.0	
20s	1915	28.6	34.5	Freshly ground.
21s	1915	67.8	78.0	Do.
21sr	1915	78.4	77.7	After standing.

[1] Expressed in cubic centimeters of normal alkali required to neutralize the extract from 1,000 gm. of material.

TABLE II.—*Further comparisons between the ice-water and the alcohol method, showing increase in acidity which takes place in ground cereals upon standing*—Continued

Sample No.	Crop year	Schindler's alcohol extraction method.	Ice-water extraction method.	Remarks.
Sulphured oats—Contd.				
43	1915	33.8	36.5	Freshly ground.
44	1916	29.8	15.0	Do.
45	1916	35.3	31.7	Do.
46	1916	33.4	36.0	Do.
47	1915	45.6	18.3	Do.
48r	1915	97.4	31.1	After standing.
49	1915	37.5	39.5	Freshly ground.
50	1915	40.0	22.7	Do.
Unsulphured barley:				
51	?	14.8	10.0	Do.
52	?	24.8	22.0	Do.
Sulphured barley:				
51s	?	21.5	18.0	Do.
52s	?	21.4	32.0	Do.
Yellow corn meal (whole kernel):				
53	?	26.4	69.5 (27.8)	No rancid odor.
54	?	15.8	33.0 (23.4)	Do.
55	?	20.6	35.0 (19.0)	Do.
White corn meal (degerminated):				
56	?	48.2	21.5 (22.0)	Do.
57	?	46.4	22.0 (21.8)	Do.

While in the work recorded in Table I all oat samples had been freshly ground before being extracted, a number of the samples listed in Table II had been ground about eight months before being handed to Mr. Mastin for analysis. These old samples gave off a more or less rancid odor, and some of them had become infested with molds. Samples of this type are marked in the table by adding the letter "r" to the original number and also by the descriptive remark in the last column. In some cases the figures obtained for the same oats when freshly ground are also given. In general, the values connected by brackets refer to the same sample of oats. Thus for sample 23 the ice-water value 8.5 was obtained for the freshly ground oat; the corresponding figure, after the sample had been ground and kept in a glass jar for several months, was 20.2; and for another sample which was moldy and more strongly deteriorated the value was 43.0. In the series of sulphured samples those marked with the letter "s" correspond to samples with the same number in the unsulphured set. In other words, a sample of a given lot of oats or barley was taken both before and after sulphuring; so the values listed under 17 are the values for the oat of that number before sulphuring, and those under 17s are the values for the same oat after it had been sulphur bleached.

Samples of the sulphured oats No. 18s and 21s which had been kept for a long time after grinding were also available for investigation,

and the acidity values found are listed under No. 18sr and 21sr, respectively. It is noticeable that these sulphured samples showed no increase in the ice-water acidity even though they had stood for a prolonged period after being ground. It would seem, therefore, that the increased titration values found in these instances with the Schindler method are wholly due to the formation, on standing, of amphoteric protein deterioration products. The latter are not determined by the ice-water method, since by its use only substances are measurable which react acid in aqueous solution. In other words, the interesting fact is apparent from these results that with ground samples which prior to the grinding have been sulphur bleached the ice-water acidity does not increase on standing, while unsulphured oats with the same method and under like conditions always show an increase in acidity. The sulphur bleaching, therefore, has probably destroyed the acid-forming ferments of the grain. It has not, however, stopped the decomposition of the proteins, and the rate of this decomposition is doubtless registered by the increased titration values obtained for these samples when Schindler's method is used. The latter method might, therefore, under certain conditions and when used in conjunction with a true acidity method, be developed into a useful measure for the rate of decomposition of proteins, as already pointed out in my previous paper.

The barley samples and many of the oats samples listed in Table II had been collected by me at various grain elevators in the Central West during the fall of 1916. The five samples of commercial corn meal No. 53 to 57 had been examined by me seven months previously by means of the ice-water method. The values then obtained are recorded in parenthesis for comparison. These corn meal samples were extracted with ice water for 1½ hours, as against 1 hour for barley and oats. The figures in Table II represent cubic centimeters of normal alkali which were required to neutralize the extract from 1,000 gm. of the material. The moisture content of the latter was not taken into account. The determinations were made in the laboratory, using an ice-water bath, in the manner described under the next heading.

The values obtained by the two methods in the extraction of the corn meals 53 to 57, which had been purchased in the open market, are also of interest.[1] The ice-water values of Mr. Mastin, compared with those which I had obtained seven months previously (the latter being shown in parenthesis) indicate a distinct rise in acidity for the whole kernel meals 53 to 55 but no rise for the degerminated meals 56 and 57. Possibly the latter meals had received some kind of chemical treatment which prevented the increase in acidity in a similar manner as the sulphur bleaching had prevented it in the oat samples 18sr and 21sr. Another explanation would be that the acid-forming ferments are all located in the germs and had been removed with the latter. Unfortunately I had

[1] The yellow corn meals 53 to 55 had been reground to about the same fineness as samples 56 and 57.

employed only the ice-water method, and not the alcohol method, when first examining these corn meals. From the figures obtained by Mr. Mastin it is also clear that by extracting samples 53 to 55 with alcohol in the manner prescribed by Besley and Baston, it was not possible to bring into solution all of the acid-reacting constituents of these meals.

FURTHER STUDY OF THE ICE-WATER EXTRACTION METHOD

The following experiments were carried out by me for the purpose of studying more closely the conditions and limitations of the ice-water extraction method.

It was thought that a brief boiling might possibly give some indication of the character of the free acids present in ice-water extractions of oats and corn. Equal aliquots of filtered ice-water extracts were therefore pipetted into Erlenmeyer flasks and the one sample heated to boiling on an asbestos screen, while the other was titrated directly. The boiling was continued for 30 seconds, whereupon the flask was removed from the screen, cooled under the tap, and at once titrated.

TABLE III.—*Effect of heating upon the acidity of ice-water extractions of oats and corn* [1]

Sample No.	Titrated directly.	Titrated after boiling ½ minute.
Unsulphured oats:		
58	20.0	19.3
59	21.0	20.0
Sulphured oats:		
58s	50.3	50.7
59s	27.3	26.0
Corn meals:		
60	55.0	55.7
61	30.7	29.3

[1] Expressed in cubic centimeters of normal alkali required to neutralize the extract from 1,000 gm. of material.

It is seen from the foregoing table that the short heating had no appreciable influence on the titration values of these samples. The boiled liquids, moreover, showed no precipitate or other visible changes. Upon standing in the cold-storage room over night both the boiled and the unboiled extracts had turned acid again. It may be mentioned that I have regularly observed that ice-water extractions of oats or corn when filtered and kept at a temperature of 1° to 2° C. for over a day without being neutralized show no change in acidity. However, if such extracts are neutralized they form fresh acid upon standing and continue to do so after renewed neutralization. The presence of chloroform does not affect this acid formation. Apparently, therefore, the acidity found in each of these extracts represents a chemical equilibrium, which tends to become reestablished if disturbed by the process of neutralization.

The acidity figures in Table IV will serve to illustrate these relations. They are also intended to show the influence of the time of extraction upon the acidities of the extracts of corn and oats. Three portions of 12 gm. each of finely ground oats were extracted with 100 cc. of ice water, the bottles being shaken at 15-minute intervals. Three 12-gm. portions of a commercial corn meal were extracted in the same manner. All six extractions were started at the same time. At the end of 1 hour the contents of one bottle each of the oat and corn infusions were poured upon dry folded filters, and two 25-cc. aliquots of the filtrate pipetted into Erlenmeyer flasks. The one aliquot was titrated at once with *N/10* sodium hydroxid, while the other aliquot was left in the cold-storage room for 24 hours before being titrated. The former aliquot, after being neutralized, was returned to the cold storage. At the end of 1½ and 2 hours, respectively, the filtration of another infusion of both corn and oats was begun, and the respective aliquots titrated in the manner above described. Together with the titration of those aliquots which had stood for 24 hours before being neutralized the aliquots neutralized on the previous day were retitrated. The alkali thus required was due to the fresh acid which had been formed after the extracts were neutralized These increments are listed in separate columns of the following table.

TABLE IV.—*Relation between time of extraction and acidity of ice-water extracts of oats and corn, and the formation of fresh acid after neutralization of the extracts* [1]

Time of extraction (in minutes).	Treatment of extract.	Oats.		Corn meal.	
		First titration.	Second titration.	First titration.	Second titration.
60	Titrated at once	0.66	0.21	0.73	0.24
	Titrated after 24 hours	.65	.....	.73	.....
90	Titrated at once	.69	.22	.86	.23
	Titrated after 24 hours	.68	.....	.85	.....
120	Titrated at once	.71	.21	.88	.21
	Titrated after 24 hours	.72	.....	.88	.....

[1] Expressed in cubic centimeters of *N/10* sodium hydroxid required to neutralize 25 cc. of extract (3 gm. of material).

It is seen from the foregoing table that the amount of acid found in the extracts varies with the time of extraction. However, with oats only slight increases in acidity were observed if the extraction period was extended beyond 1 hour. With corn, the difference between the values for the 60- and 90-minute extractions was rather marked, whereas the difference between the 90- and 120-minute extractions was insignificant.

There are two possible explanations for the slight differences in the quantity of acids found in the three oat extracts of Table IV. One would be that the acids present in the material are dissolved very slowly

and the other that the total original acidity of this oat is included in the value found for the 60-minute extraction and that the rise upon prolonged extraction is due to ferment action. From my present experience I would prefer the latter explanation. While, as shown above, no acid-producing ferments come into play in the filtered, unneutralized grain extracts at the cold-storage temperature, it is quite conceivable that if the liquid remains in prolonged contact with the grain solids some insoluble constituents may be hydrolized by specific ferments and form acid-reacting, soluble cleavage products.

It would be difficult to understand why the total acids originally present in oats should not dissolve within 1 hour and hence should not be included in the 60-minute acidity value, in view of the fact that, as Thatcher and Koch (*16*) have shown, all of the diastase of cereal products can be brought into solution by a 1-hour extraction with ice water. Diastase, being a colloid, is considered soluble with some difficulty, while acid-reacting substances which occur in nature are known to be readily soluble in water.

From the corn meal acidities recorded in Table IV it would appear that for corn meals the extraction period in the ice-water method should be longer than for oats. For the experiment I used a commercial white corn meal which was sufficiently fine to be passed through a 20-mesh sieve. Notwithstanding this fineness slightly more than 1 hour's time appeared to be required to dissolve with ice water all of the acid contained in this meal. It follows that the extraction period should be extended to 1½ hours for corn possessing this degree of fineness.

On the whole it may be said that the ice-water extraction method affords a very rapid and inexpensive means for determining the original quantity of free acids contained in cereal products, and the method is no doubt of wide applicability. The results obtained by its use are sufficiently accurate for practical purposes. At the same time it permits the experimenter to devise such modifications as may suggest themselves in special cases. For practical routine work the use of a cold-storage room can, of course, be easily dispensed with by immersing the tightly stoppered extraction bottles in a vessel with ice water. In the work of Mr. Mastin, who, as stated, is responsible for the acidity figures recorded in Table II, this mode of operation was employed. It was also found preferable to use a *N/20* solution of sodium hydroxid for the titrations. In general, the following suggestions for the successful operation of the method may be given:

APPARATUS AND REAGENTS REQUIRED

1. A flat-bottomed pan, with walls at least 12.5 cm. high, which is kept filled with ice water.
2. Extraction bottles of ordinary glass, provided with well-fitting rubber stoppers.
3. Graduated cylinders, capacity 100 cc.
4. Glass funnels.
5. Wide-mouthed beaker flasks, capacity 100 cc.
6. Folded filter paper.

7. A volumetric pipette, capacity 25 cc.
8. A volumetric pipette, capacity 150 cc.
9. An alcoholic solution of phenolphthalein (0.15 to 1 per cent strength).
10. A *N/20* solution of sodium hydroxid.
11. A supply of distilled water, previously freed from carbon dioxid by boiling, kept on hand in the refrigerator or immersed in ice water.

PREPARATION OF SAMPLE

All materials to be tested for acidity should be in a finely divided state in order to make possible a complete and rapid extraction of the acids. It is also to be borne in mind that cereal products usually undergo rapid changes in acidity after they have once been ground.

METHOD OF MAKING THE TEST

Weighed quantities of the finely ground materials, for example 15 gm. of each sample, are placed in the dry extraction bottle, and a tenfold quantity of the precooled distilled water is pipetted into each of the bottles. The latter are now stoppered tightly, well shaken, and immersed in the flat-bottomed pan holding the ice water. At 15-minute intervals they are momentarily removed from the bath to be shaken vigorously. Unless this is done at the prescribed intervals, or oftener, accurate results will not be obtained. While the extractions are in progress preparations may be made for the filtrations. At the end of the extraction period (1 hour for oats, 1½ hours for corn) the mixtures are immediately thrown upon dry folded filters, the filtrates being collected in the graduated cylinders. The first few cubic centimeters of each filtrate may be discarded. If filtration is slow, or during hot weather, it is best to keep the filtrates cool by placing the graduated cylinder in the pan with ice water during filtration. A measured aliquot, for example 25 cc. of each filtrate, is finally pipetted into a beaker flask, and titrated with the alkali, after adding a few drops of phenolphthalein. It has been customary in this country to express the acidity in terms of cubic centimeters of normal alkali required to neutralize the acid extracted from 1,000 gm. of the material. For very accurate work allowance would have to be made in the calculations for the volume occupied by the undissolved portion of the material, but for practical purposes this correction may be omitted.

The titration in the presence of phenolphthalein offers no difficulties, except in cases where the grain product is strongly damaged and discolored. In such cases the filtered extracts may show a deep yellow color, and the endpoint of titration becomes difficult to recognize. This difficulty, if met with, can be overcome by the use of colorimetric devices, such as the apparatus employed by Lüers and Adler (*4,10*).

ACIDITY OF SOUND OATS OF PURE VARIETIES DETERMINED BY THE ICE-WATER EXTRACTION METHOD

It seemed of interest to examine by means of the ice-water method a number of sound oats of known type and origin, and to compare their acidities with those found for the more or less damaged oats listed in Table I. Samples of pure strains of oats were secured from various experiment farms of the Department of Agriculture through the Office of Cereal Investigations, Bureau of Plant Industry. Two samples

received from the individual growers in the central coast region of California and known to be of high quality were included in this experiment. The titration results, calculated to the basis of dry weight, are given in the following table.

TABLE V.—*Acidity values of different varieties of oats shown by the ice-water method*[1]

Sample No.	Variety.	Locality.	Crop year.	Acidity value.
62	Green Russian	Iowa	1914	16.30
63	Silvermine	do	1914	23.00
64	Canadian	Idaho	1915	22.00
65	Silvermine	do	1915	24.00
66	Swedish Select	North Dakota	1915	13.50
67	do	South Dakota	1915	15.75
68	Winter Turf	Virginia	1915	17.50
69	Red Rustproof	do	1915	20.00
70	Sixty Day	South Dakota	1915	13.60
71	Victory	North Dakota	1915	14.00
72	Abundance	do	1915	15.50
73	Siberian	do	1915	19.90
74	Red Oats	California	1914	8.75
75	Black Oats	do	1914	10.50

[1] Expressed in cubic centimeters of normal alkali required to neutralize the extract from 1,000 gm. of material.

If we compare the above acidity values with each other and with those of the unsulphured but damaged oats in Table I, we find a remarkable degree of uniformity, considering that the samples represent oats of two seasons and grown in different localities. We observe, above all, no essential difference in the values for the damaged oats (in Table I) from those of the sound oats (in Table V). This clearly shows that the amount of free acid present in oats does not change materially in the unground kernel during the early stages of spoilage. The lowest acidities found in sound oats were those of the two samples from the Pacific coast, and in no case was the value as high as 25. Incidentally, there are indications that certain constant acidity values are characteristic of the different varieties. Thus the values for samples 66 and 67 in Table V, which are both of the Swedish Select variety and had been grown in different States, are not far apart. Similarly, No. 63 and 65 of the Silvermine variety show nearly the same acidity, although grown in different States and seasons.

SUMMARY

(1) Various deficiencies of the Schindler method for measuring the acidity of cereal products are pointed out. These deficiencies are attributable to the presence of alcohol during the extraction and subsequent titration.

(2) A new and simple method is recommended for determining the amount of free acid originally present in cereal products. The im-

portant feature of the new method is the use of ice water for the extraction of the material.

(3) By means of the ice-water method it is shown that the amount of acid present in oat kernels does not change markedly during the early stages of spoilage. If oats are sulphured their acidity is increased.

(4) Oats which previously had been sulphur bleached showed no increase in acidity upon prolonged standing in the ground state when tested by means of the ice-water method, the acid-forming ferments of the grain having been destroyed by the sulphur fumes. With Schindler's method pronounced increases in the titration values were still observed in these cases, owing doubtless to the fact that certain protein cleavage products continue to be formed, which in aqueous solution are amphotheric, but which possess an acid reaction in the presence of alcohol.

(5) Ice-water extracts of oats or corn, if filtered and kept at the temperature of 1° to 2° C. for 24 hours without being neutralized, undergo no change in acidity. If neutralized, a new formation of acid takes place, notwithstanding the low temperature.

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RATE OF ABSORPTION OF SOIL CONSTITUENTS AT SUCCESSIVE STAGES OF PLANT GROWTH

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Data derived from the periodic cuttings of a crop of barley in 1916 were used in a previous paper (*1*)[2] to develop certain general relations between the amounts of soil constituents acquired by the plant at various stages of growth and those present in the water extracts of soils. It is evident that the value of conclusions drawn from such data is somewhat limited if based upon results obtained from a single soil. On the other hand, the detailed and laborious studies necessary in such inquiries may not be indefinitely repeated; and further work of this kind was not originally contemplated. In the course of the work referred to, however, certain aberrations in the data appeared to indicate errors or to lead to conclusions of such an extraordinary character as to demand additional experimental verification, so a further study was carried out the following season, 1917. The principal object of this paper is to present the result then obtained. Since, however, these justify the data from the earlier work and corroborate certain important conclusions logically deducible therefrom, but not hitherto published, the complete results of the periodic harvests from both studies will be considered.

CONDITIONS OF THE EXPERIMENTS

The details of the experiments were formulated primarily to insure:

That the individual plants in each experiment should have access to an equal volume of soil.

That there should be no opportunity for loss of soil constituents by drainage or leaching, or gain from the constituents of rain or irrigation water.

That there should be no opportunity for removal of plant constituents by the washing off of effloresced or soluble constituents by rain.

[1] Thanks are extended to Messrs. A. W. Christie and J. C. Martin for performing the analyses reported herein and for assistance in the computations, etc., and to Prof. G. R. Stewart for supervision of the cultural arrangements and harvesting of the plants.

[2] Reference is made by number (italic) to "Literature cited," p. 72.

The crop was the selected strain of Beldi barley used in much of the previous work of this laboratory. The plants were grown on a silty clay loam soil (*1c*) in 1916 and on a fine sandy loam soil (*15*) in 1917.

In the 1917 experiment the soil, made as homogeneous as possible by sifting and mixing, was installed in 8 tight galvanized iron boxes or containers with a surface area of 30 by 60 inches each and a depth of 18 inches. Two of the containers were equally divided by a lateral partition, giving a total of 10 independent compartments. The soil in one of the large containers was used as a control plot, and the other plots were used for growing the crop. The plants were placed 6 inches apart in the row with 6 inches between rows. Thus there were 50 plants to each of the large containers and 25 plants to each of the small containers, with equal volumes, weights, and superficial areas of soil per plant. The plants were cut at intervals of two weeks throughout the season, beginning with the third week from planting. At the same time samples were drawn and water extractions made from soil in the control plot container and from the containers being cropped. No further soil samples were taken from any container after the plants were removed therefrom. The containers were compactly arranged, insulated from heat fluctuations except at the surface, and protected from birds and rodents. All plots were watered with distilled water only and protected during the infrequent rains by rubber covers placed on wire frames. The mechanical arrangement and methods of sampling soils were in all respects the same as those described in greater detail in connection with other experiments from this laboratory (*8*). In order to obtain sufficient material for the chemical studies, we used the contents of two of the large containers for the first cutting, one large container for each of the three succeeding cuttings, and one small container each for the last four cuttings. The minimum number of plants embraced by any cutting was 23 at the fifteenth week from planting, in which instance 2 plants failed to develop after thinning.

The procedure in the 1916 experiment was similar to that of 1917. A minor difference which should be noted was that the containers for the cropped soil in 1916 were of wood and varied several inches in each dimension from the iron containers used in the work of the following year. A more important difference lies in the fact that in attempting to economize equipment and soil, which was brought from a distance at considerable expense, we used two containers of soil, one cropped and one uncropped, which had been in place and were grown to a crop of barley in the year 1915. One of these was used as a control plot and one for the determination of total yield at the end of the season. Subsequent determinations of the water-extractable matters from these soils showed somewhat higher concentrations than those from the soils in the newer containers from which the successive cuttings were made. This could have had no effect on the data obtained for the plant, inasmuch as the periodic cuttings were all made on soil assembled, mixed, and installed

at one time, but may be regarded as vitiating the data obtained from the soils for certain other purposes. To obviate criticism on this point we shall in the present paper confine our conclusions as to the soil to those obtained from the data of the 1917 experiment, although these are in all general relations confirmed by the work of the preceding year.

CHEMICAL TECHNIC IN THE EXPERIMENTAL WORK

The water extractions of the soils and the analyses of the extracts were made by the methods described by Stewart (*8*); the analyses of the plants were made by the usual methods of treatment for nitrogen and plant ashes, with proper precautions to prevent the loss of partially volatile constituents.

MECHANICAL DIVISION OF PLANTS

Since the object of the investigation was to bring out certain general relations between the type crop and the soil, it was not deemed desirable or profitable to make a complete separation of the plants based on their more minute anatomical structure. The well-known and substantial differences in composition between the heads of grain crops and the vegetative tissues seemed, however, to require the separation of the plant into at least these two parts; furthermore the roots obviously deserve separate consideration. The procedure actually followed was to remove the entire plant with as much of the roots as possible. In all cuttings after the heads had formed these were separated from the vegetative portion, designated stems and leaves. The roots of all plants were severed by a lateral cut through the base of the crown. As separated, this portion of the plants therefore included a small portion of the lower part of the crown. The roots so recovered included only a small proportion of the finer rootlets. Most of these had broken loose from the plants and were to be found throughout the soil in very fine and fragile strands which it is hopeless to expect to remove in their entirety.

The results reported hereafter on the upper part of the plant make it appear probable that a study of root composition would give important data on the mechanism of plant absorption, but that such a study should be based on very exact measurements of the quantities and composition of the roots. For this purpose natural soils, even when the roots are washed free, do not appear to offer the best medium for growing the plants, inasmuch as the data would always be subject to the criticism that the recovery was incomplete, or that the roots had sustained losses of solutes from the washing or gains from adhering soil particles. On this account we abandoned the idea of making a chemical study of the roots collected in the plot experiments, but will present evidence from other sources as to the effect of root composition on our conclusions.

PRESENTATION OF DATA

As a matter of record the original data are given in terms of yields and of the composition of the moisture-free material. (See Tables I and II.)

All figures are, however, recomputed in terms of the absolute amounts of the constituents reported. These are expressed both as grams per plant and as parts per million of soil. (See Tables III and IV.)

Inasmuch as the succeeding discussion centers about the graphs, presented in some detail, a word concerning these is perhaps necessary. The 2-week interval between cuttings, used for the most part, appears to have been sufficiently short to bring out the more important characteristics of growth and absorption. It will be noted, however, that in the 1916 experiment the first cutting did not take place until six weeks after planting. The fact that the plants did not appear above ground for several weeks and the further fact that the earlier harvests of the 1917 experiment showed very characteristic changes, indicate that the use of a straight line to cover this period in the 1916 graphs is highly artificial. However, the method is consistent in that we have connected only known points; and the facts as to absorption and growth at this stage are sufficiently clarified by the 1917 experiment.

Since our data are derived from two relatively independent studies of the growth of barley on two very different soils in two different calendar years, it is obvious that differences may be expected to appear which are equally assignable to one or another of the conditions of growth such as soil, season, etc. Our present purpose is not to account for such differences, but to call attention to certain similarities which are rendered all the more striking in view of the great differences in the yields actually obtained.

TABLE I.—*Barley at successive stages of growth*

GROWN ON SILTY CLAY LOAM SOIL (1C) IN SEASON OF 1916

Date.	Age from planting (in weeks).	Number of plants.	Description.	Average weight per plant (in grams).			Percentage of moisture.	Percentage of dry matter.	Analyses of moisture-free material.				
				Total.	Moisture.	Dry matter.			Percentage of nitrogen.	Percentage of phosphorus.	Percentage of potassium.	Percentage of calcium.	Percentage of magnesium.
June 12	6	69	Entire, except roots [a]	23.84	21.62	2.22	90.70	9.30	3.71	0.50	5.40	0.47	0.54
26	8	49	.....do	47.96	39.82	8.14	83.02	16.98	1.66	.41	3.70	.37	.34
July 10	10	42	.....do	48.83	36.73	12.10	75.23	24.77	.91	.29	1.87	.25	.27
24	12	29	.....do	48.66	32.80	15.86	67.40	32.60	.77	.25	1.23	.23	.25
Aug. 7	14	28	.....do	47.43	30.21	17.22	63.70	36.30	.76	.26	1.17	.19	.22
21	16	38	.....do	29.45	9.53	19.92	32.35	67.65	.71	.28	1.24	.22	.22
28	17	87	.....do	23.15	3.00	20.15	16.96	87.04	.71	.28	1.19	.18	.22
July 10	10	42	Stems and leaves	42.12	32.08	10.04	76.14	23.86	.80	.29	2.09	.28	.29
24	12	29	.....do	32.55	23.38	9.17	71.82	28.18	.45	.16	1.71	.34	.31
Aug. 7	14	28	.....do	30.04	21.79	8.25	72.53	27.47	.27	.10	1.89	.33	.28
21	16	38	.....do. [b]	15.58	3.84	11.74	24.66	75.34	.27	.15	1.77	.33	.27
28	17	87	.....do. [b]	13.04	1.42	11.62	10.84	89.16	.24	.13	1.74	.28	.24
July 10	10	42	Heads	6.72	4.67	2.05	69.50	30.50	1.30	.32	.81	.12	.15
24	12	29	.....do	16.10	9.41	6.69	58.46	41.54	1.22	.37	.57	.07	.18
Aug. 7	14	28	.....do	17.40	8.43	8.97	48.46	51.54	1.22	.40	.51	.06	.17
21	16	38	Grain	13.87	5.68	8.19	41.00	59.00	1.34	.47	.49	.05	.15
28	17	87	.....do	10.12	1.59	8.53	15.88	84.12	1.36	.49	.44	.05	.18

[a] See subsequent data and discussion concerning effect of omitting roots.

[b] Stems and leaves include chaff from grain on this date.

TABLE II.—*Barley at successive stages of growth*

GROWN ON SANDY LOAM SOIL (15) IN SEASON OF 1917

Date.	Age from planting (in weeks).	Number of plants.	Description.	Average weight per plant (in grams).			Percentage of moisture.	Percentage of dry matter.	Analyses of moisture-free material.				
				Total.	Moisture.	Dry matter.			Percentage of nitrogen.	Percentage of phosphorus.	Percentage of potassium.	Percentage of calcium.	Percentage of magnesium.
May 21	3	97	Entire, except roots[a]	0.7630	0.6867	0.0763	90.00	10.00	6.16	0.79	6.16	0.92	0.92
June 4	5	49	do	6.652	5.879	.773	88.40	11.60	5.51	.60	5.96	.78	.47
18	7	50	do	33.140	29.042	4.098	87.59	12.41	3.27	.55	4.87	.59	.41
July 2	9	49	do	81.80	70.01	11.79	85.59	14.41	2.58	.35	3.51	.52	.31
16	11	25	do	76.80	58.56	18.24	76.09	23.91	1.04	.27	1.50	.29	.17
30	13	25	do	85.88	58.09	27.79	67.64	32.36	.87	.25	1.15	.28	.14
Aug. 13	15	23	do	107.90	71.17	36.73	65.95	34.05	.97	.24	1.21	.29	.18
Sept. 6	18+	25	do	57.60	28.17	29.43	48.91	51.09	1.22	.31	1.22	.31	.18
July 2	9	49	Stems and leaves	78.85	67.755	11.095	85.93	14.07	2.62	.35	3.63	.55	.32
16	11	25	do	66.72	51.63	15.09	77.38	22.62	.93	.26	1.61	.32	.18
30	13	25	do	63.36	44.34	19.02	69.98	30.02	.61	.20	1.36	.36	.14
Aug. 13	15	23	do	70.78	49.16	21.62	69.46	30.54	.47	.12	1.70	.43	.18
Sept. 6	18+	25	do[b]	38.84	23.18	15.66	59.68	40.32	.50	.12	1.94	.57	.23
July 2	9	49	Heads	2.939	2.247	.692	76.46	23.54	1.98	.38	1.52	.12	.15
16	11	25	do	10.08	6.938	3.142	68.84	31.16	1.58	.31	1.00	.15	.13
30	13	25	do	22.52	13.752	8.768	61.07	38.93	1.44	.36	.70	.11	.13
Aug. 13	15	23	do	32.74	17.63	15.11	53.85	46.15	1.69	.42	.50	.09	.17
Sept. 6	18+	25	Grain	18.76	3.90	14.86	20.79	79.21	1.92	.49	.51	.05	.13

[a] See subsequent data and discussion concerning effect of omitting roots.

[b] Stems and leaves include chaff from grain on this date.

TABLE III.—*Constituents of barley withdrawn from a silty clay loam soil (1c) in 1916*

Date.	Age from planting. (in weeks).	Number of plants.	Description.	Nitrogen (grams per plant).	Nitrogen[a] as nitrate (parts per million of soil).[b]	Phosphorus (grams per plant).	Phosphorus[a] as phosphate (parts per million of soil).[b]	Potassium (K).		Calcium (Ca).		Magnesium (Mg).	
								Grams per plant.	Parts per million of soil.[b]	Grams per plant.	Parts per million of soil.[b]	Grams per plant.	Parts per million of soil.[b]
June 12	6	69	Entire, except roots	0.0824	22.36	0.0111	2.09	0.1199	7.35	0.0104	0.64	0.0120	0.74
26	8	49	.....do	.1351	36.67	.0334	6.28	.3012	18.47	.0301	1.85	.0278	1.71
July 10	10	42	.....do	.1097	29.77	.0357	6.71	.2264	13.49	.0306	1.88	.0322	1.97
24	12	29	.....do	1229	33.35	.0394	7.40	.1949	11.95	.0359	2.20	.0404	2.48
Aug. 7	14	28	.....do	1317	35.74	.0443	8.32	.2017	12.37	.0326	2.00	.0384	2.35
21	16	38	.....do	1414	38.37	.0561	10.54	.2479	15.20	.0428	2.62	.0440	2.70
28	17	87	.....do	1439	39.05	.0569	10.69	.2397	14.70	.0368	2.26	.0433	2.66
July 10	10	42	Stems and leaves	083	22.53	.0291	5.47	.2098	12.86	.0281	1.72	.0291	1.78
24	12	29	.....do	0413	11.21	.0147	2.76	.1568	9.62	.0312	1.91	.0284	1.74
Aug. 7	14	28	.....do	0223	6.05	.0083	1.56	.1559	9.56	.0272	1.67	.0231	1.42
21	16	38	.....do.[c]	.0317	8.60	.0176	3.31	.2078	12.74	.0387	2.37	.0317	1.94
28	17	87	.....do.[c]	.0279	7.57	.0151	2.84	.2022	12.40	.0325	1.99	.0279	1.71
July 10	10	42	Heads	.0267	7.25	.0066	1.24	.0166	1.02	.0025	.15	.0031	.19
24	12	29	.....do	.0816	22.15	.0247	4.64	.0381	2.34	.0047	.29	.0120	.74
Aug. 7	14	28	.....do	.1094	29.69	.0360	6.77	.0458	2.81	.0054	.33	.0153	.94
21	16	38	Grain	.1097	29.77	.0385	7.24	.0401	2.46	.0041	.25	.0123	.75
28	17	87	.....do	.1160	31.49	.0418	7.85	.0375	2.30	.0043	.26	.0154	.94

[a] Nitrogen and phosphorus are here computed in terms of nitrate (NO_3) and phosphate (PO_4) for the purpose of making clearer the subsequent comparisons between plant withdrawals and the nitrate and phosphate of the soil as determined by water extraction.

[b] Computation of parts per million is based on the number of grams per plant and a unit container of 50 plants and 1,800 pounds of soil.

Grams per plant, times 61.23 $\left[\frac{50}{1{,}800\times 453}\times 1{,}000{,}000\right]$ = parts per million.

[c] Stems and leaves include chaff from grain on this date.

TABLE IV.—*Constituents of barley withdrawn from a sandy loam soil (15) in 1917*

Date.	Age from planting (in weeks).	Number of plants.	Description.	Nitrogen (grams per plant).	Nitrogen [a] as nitrate (parts per million of soil).[b]	Phosphorus (grams per plant).	Phosphorus [a] as phosphate (parts per million of soil).[b]	Potassium (K).		Calcium (Ca).		Magnesium (Mg).	
								Grams per plant.	Parts per million of soil.[b]	Grams per plant.	Parts per million of soil.[b]	Grams per plant.	Parts per million of soil.[b]
May 21	3	97	Entire, except roots	0.0047	1.351	0.0006	0.1194	0.0047	0.3052	0.0007	0.0454	0.0007	0.0454
June 4	5	49	do	.0426	12.25	.0047	.935	.0461	2.993	.0060	.3896	.0036	.2337
18	7	50	do	.1340	38.52	.0225	4.477	.1996	12.96	.0242	1.571	.0168	1.091
July 2	9	49	do	.3044	87.50	.0414	8.237	.4132	26.83	.0618	4.012	.0365	2.37
16	11	25	do	.1899	54.60	.0489	9.730	.2744	17.82	.0530	3.441	.0313	2.032
30	13	25	do	.2423	69.66	.0696	13.85	.3201	20.78	.0781	5.071	.0380	2.467
Aug 13	15	23	do	.3569	102.60	.0894	17.79	.4430	28.76	.1066	6.922	.0646	4.195
Sept. 6	18+	25	do	.3581	102.95	.0903	17.97	.3584	23.27	.0905	5.876	.0528	3.428
July 2	9	49	Stems and leaves	.2907	83.57	.0388	7.72	.4027	26.15	.0610	3.96	.0355	2.305
16	11	25	do	.1403	40.34	.0392	7.80	.2430	15.78	.0483	3.136	.0272	1.766
30	13	25	do	.1160	33.35	.0380	7.56	.2587	16.80	.0685	4.447	.0266	1.727
Aug 13	15	23	do	.1016	29.21	.0259	5.15	.3675	23.86	.0930	6.038	.0389	2.526
Sept. 6	18+	25	do[c]	.0728	20.93	.0175	3.48	.2826	18.35	.0831	5.396	.0335	2.175
July 2	9	49	Heads	.0137	3.93	.0026	.517	.0105	.682	.0008	.0519	.0010	.0649
16	11	25	do	.0496	14.26	.0097	1.93	.0314	2.039	.0047	.3052	.0041	.2662
30	13	25	do	.1263	36.31	.0316	6.288	.0614	3.986	.0096	.6233	.0114	.7402
Aug. 13	15	23	do	.2553	73.40	.0635	12.63	.0755	4.902	.0136	.883	.0257	1.669
Sept. 6	18+	25	Grain	.2853	82.02	.0728	14.48	.0758	4.922	.0074	.480	.0193	1.253

[a] Nitrogen and phosphorus are here computed in terms of nitrate (NO_3) and phosphate (PO_4) for the purpose of making clearer the subsequent comparisons between plant withdrawals and the nitrate and phosphate of the soil as determined by water extraction.

[b] Computation of parts per million is based on the number of grams per plant and a unit container of 50 plants and 1,700 pounds of soil.

Grams per plant, times 64.93 $\left[\frac{50}{1{,}700 \times 453} \times 1{,}000{,}000\right]$ = parts per million.

[c] Stems and leaves include chaff from grain on this date.

GROWTH PERIODS.—If we refer now to figures 1 and 2, it appears that growth may be divided into three periods: a preliminary period of eight to nine weeks from planting (five to six weeks from germination)

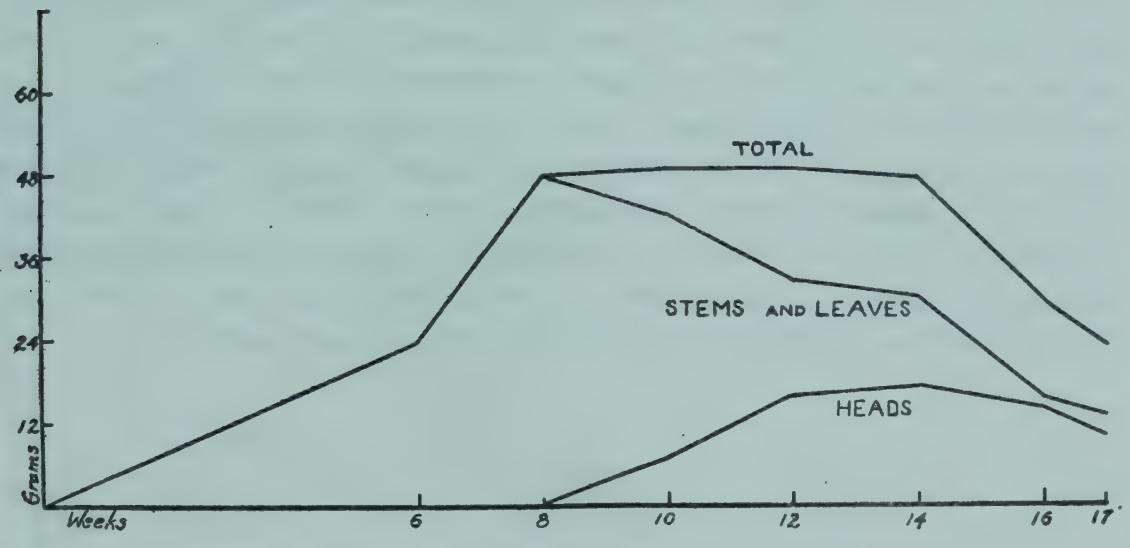

FIG. 1.—Growth reached by barley at different periods of cutting. Experiment of 1916.

in which the greatest gain in weight of the plant takes place; a second period of about six weeks, when the rate of gain in total weight falls off noticeably and in the 1916 experiment becomes negligible (it is during this period that the heads are formed and developed); and a

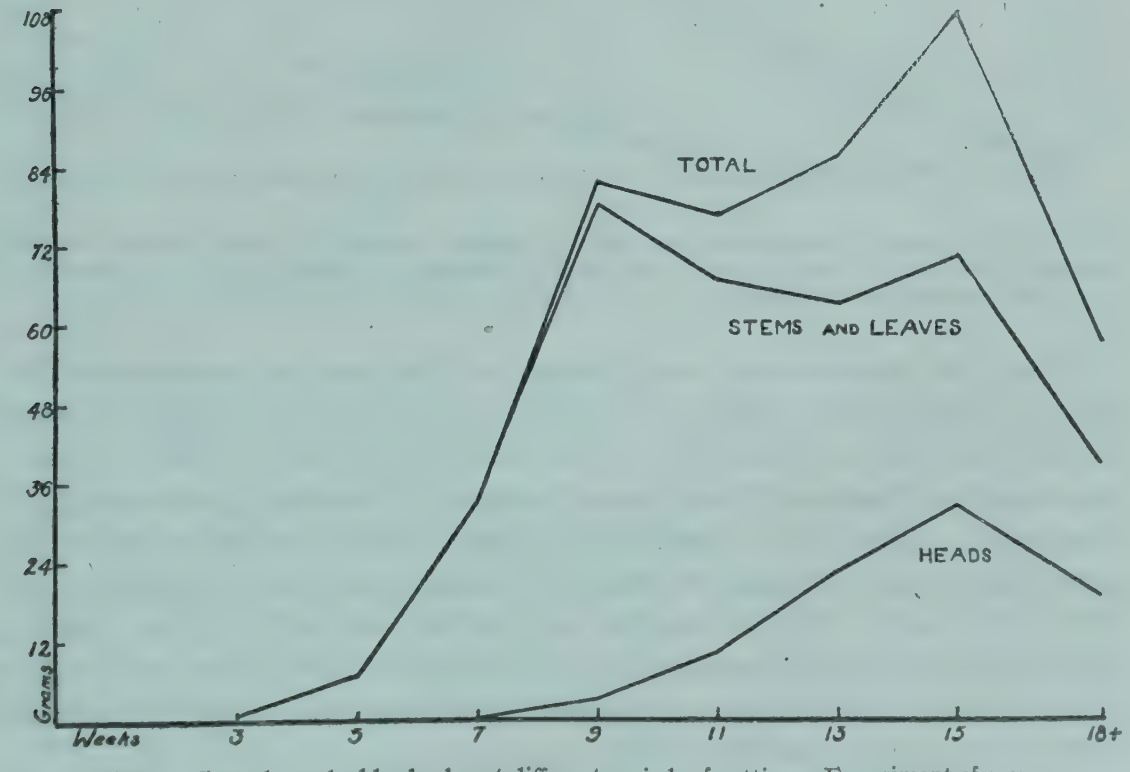

FIG. 2.—Growth reached by barley at different periods of cutting. Experiment of 1917.

third period of about three weeks, in which there is an absolute loss in weight not only in the plants as a whole but in the various parts, as is shown in both experiments. The first period is one of intense vegetative

activity; the leaves are a vivid green; the tissues are moist; and growth, measured in both weight and height, is considerable. The second period is clearly one of a different kind of activity; structural differentiation is taking place; and fully formed heads may be developed without any further increase in the growth of the plant, as is shown in the 1916 experiment when there was practically no increase in fresh weight after the eighth week. The leaves lose their green and moist appearance and the stems and leaves fall off in total weight whether that of the entire plant is increasing or not. The third period, again, is obviously different and is characterized by a loss of weight and by desiccation of all parts of the plant, accompanied by a more or less complete loss of the green color of actively growing plant tissue.

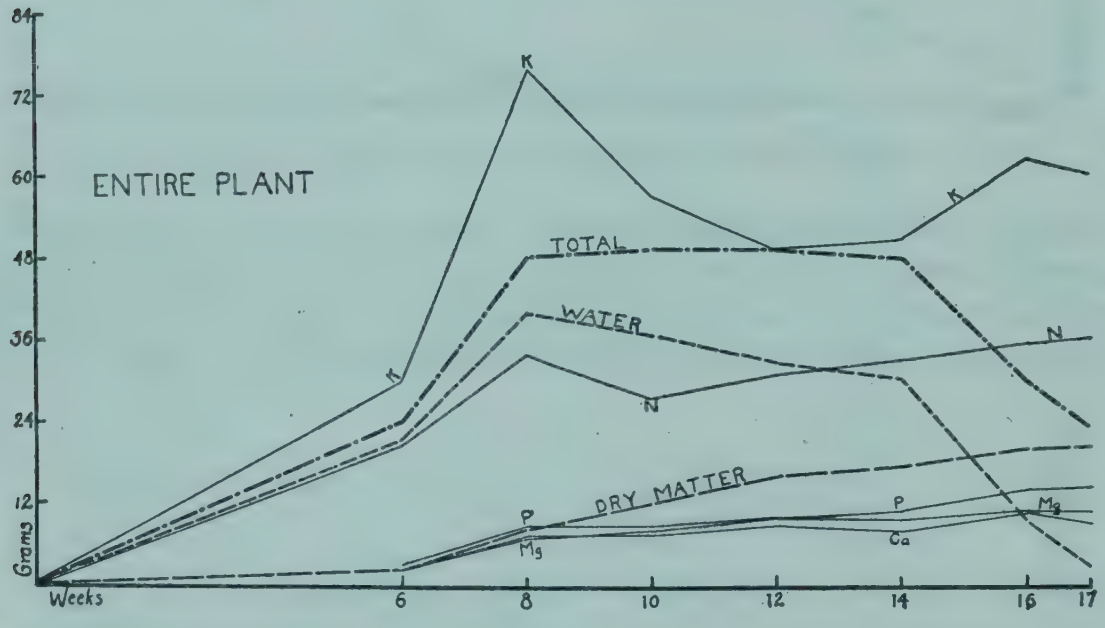

FIG 3.—Relation of growth of barley to absorption of potassium, nitrogen, phosphate, calcium, and magnesium, for entire plant except roots. To emphasize the comparative differences in absorption of these elements, the quantities actually found have been multiplied by 250. Experiment of 1916.

When we consider the composition of the plant as represented by the dry matter and water content (fig. 3, 4) no very obvious relation is observed between the dry matter and the growth periods referred to heretofore; the variation in water content is, however, quite consistent therewith. This follows naturally enough from the fact that water constitutes such a large proportion of the plant up to the last four weeks of growth. The increase in the dry-matter content of the plant is represented by a fairly straight line, the only considerable break being in the plants from the more productive soil near the end of the season when there was an absolute loss.

The water content shows considerable variations, indicated by very sharp breaks in the lines at the beginning and end of the second growth period. The first of these breaks appears to be particularly significant because the soils were being maintained at that time at constant

(optimum) water content. This loss of water points to abrupt changes in the character of the internal activities of the plant, in complete accord with the vegetative changes to which attention has been called.

ABSORPTION BY THE PLANT.—(Fig. 3–8.) The expedient we have adopted of plotting the various elements on an enlarged scale brings out important differences in their behavior. Potassium and nitrogen, both in magnitude and in rate of absorption by the plant at all stages, are more nearly proportional to the total growth and water content of the plant than to that of the dry matter, while the reverse is true of calcium, magnesium, and phosphorus.

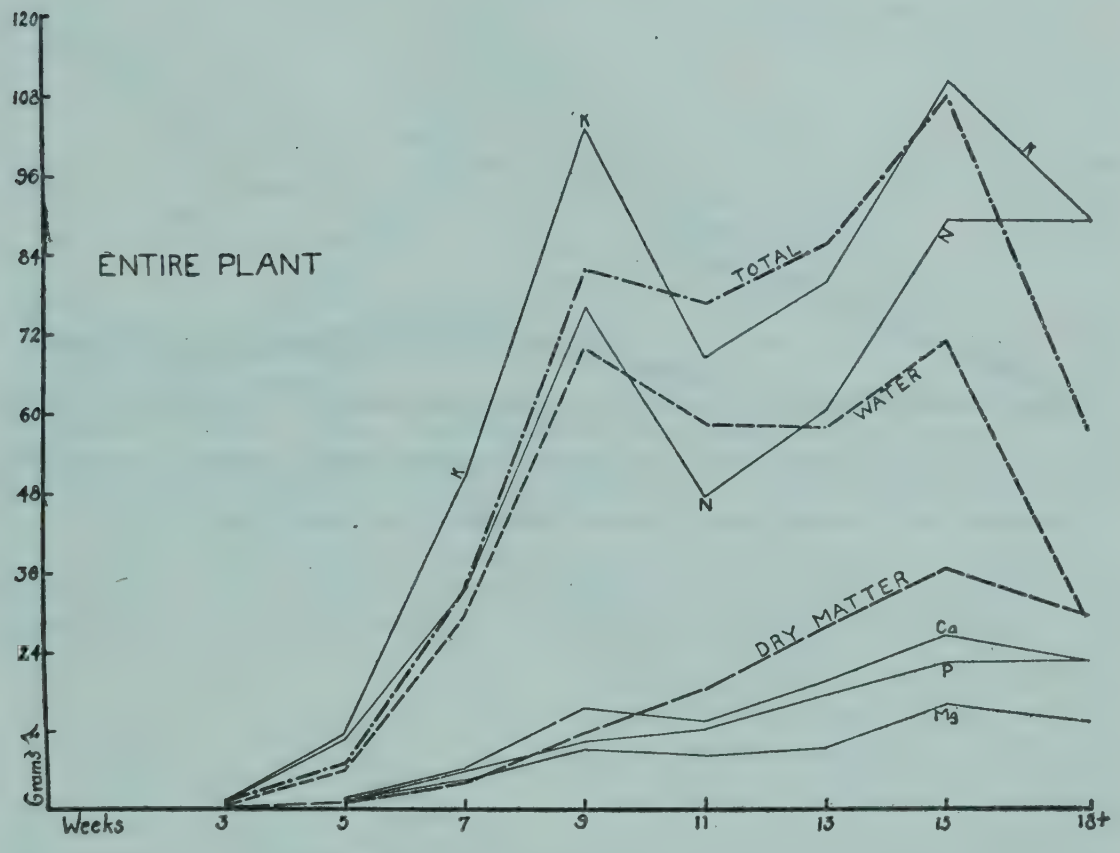

FIG. 4.—Relation of growth of barley to absorption of potassium, nitrogen, phosphate, calcium, and magnesium, for entire plant except roots. To emphasize the comparative differences in absorption of these elements, the quantities actually found have been multiplied by 250. Experiment of 1917.

During the first period of growth, ending eight and nine weeks from planting, the increase of potassium and nitrogen appears to conform very closely to the gain in total weight and water content of the plant. At the inception of the second growth period the interesting fact is developed that both of these elements diminish, apparently indicating a movement from the plant to the soil.

The increase of calcium, magnesium, and phosphorus closely parallels the formation of dry matter in both experiments up to eight and nine weeks, respectively, after which these elements lag behind. In the 1917 experiment a slight loss of calcium appears to take place between the ninth and eleventh weeks. The variation of the calcium in the

1916 experiment and of magnesium in both experiments at this same stage of growth is so small as to be within the experimental error of the

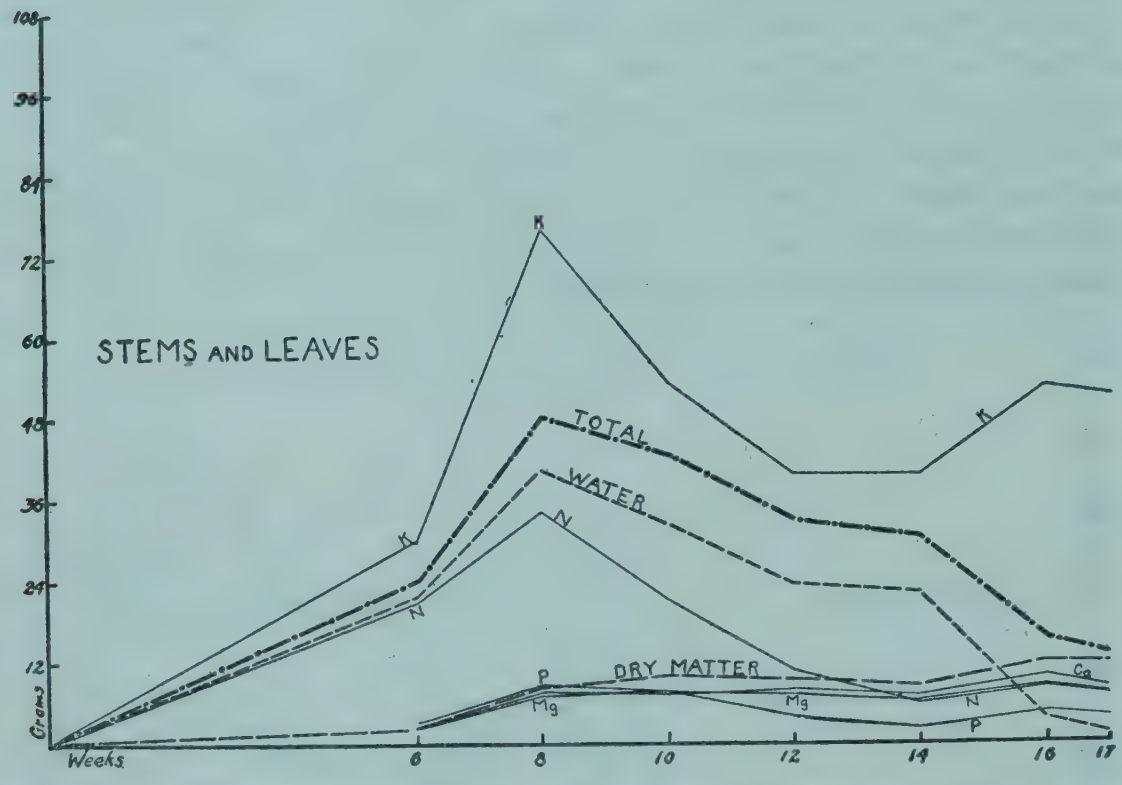

FIG. 5.—Relation of growth of barley to absorption of potassium, nitrogen, phosphate, calcium, and magnesium, for stems and leaves. To emphasize the comparative differences in absorption of these elements, the quantities actually found have been multiplied by 250. Experiment of 1916.

determination. It is quite possible, however, that the same causes that bring about such substantial losses of potassium and nitrogen at this time and appear to affect calcium, which has such different functions in plant metabolism, may not be without effect on the other elements. When the absolute losses noted above were first observed in 1916, we were inclined to doubt the accuracy of our data; but the 1917 experiment confirmed the observation in a most striking and convincing manner.

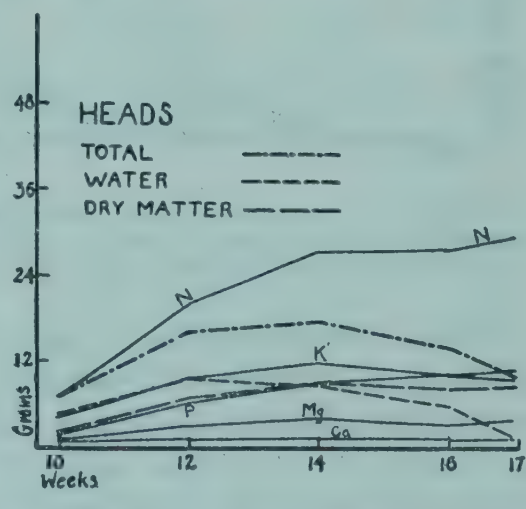

FIG. 6.—Relation of growth of barley to absorption of potassium, nitrogen, phosphate, calcium, and magnesium, for heads. To emphasize the comparative differences in absorption of these elements, the quantities actually found have been multiplied by 250. Experiment of 1916.

LOSSES OF SOIL CONSTITUENTS FROM THE PLANT.—The losses observed were not mechanical losses in decayed leaves (7), since they occurred at a period before there was any considerable drying out of the plant; furthermore the losses of fragments of vegetative tissue were negligible at all times under the conditions of the experiments. Losses from leach-

ing of the leaves, such as those suggested by LeClerc and Breazeale (*6*) and others, were rendered impossible by the protection of the plants

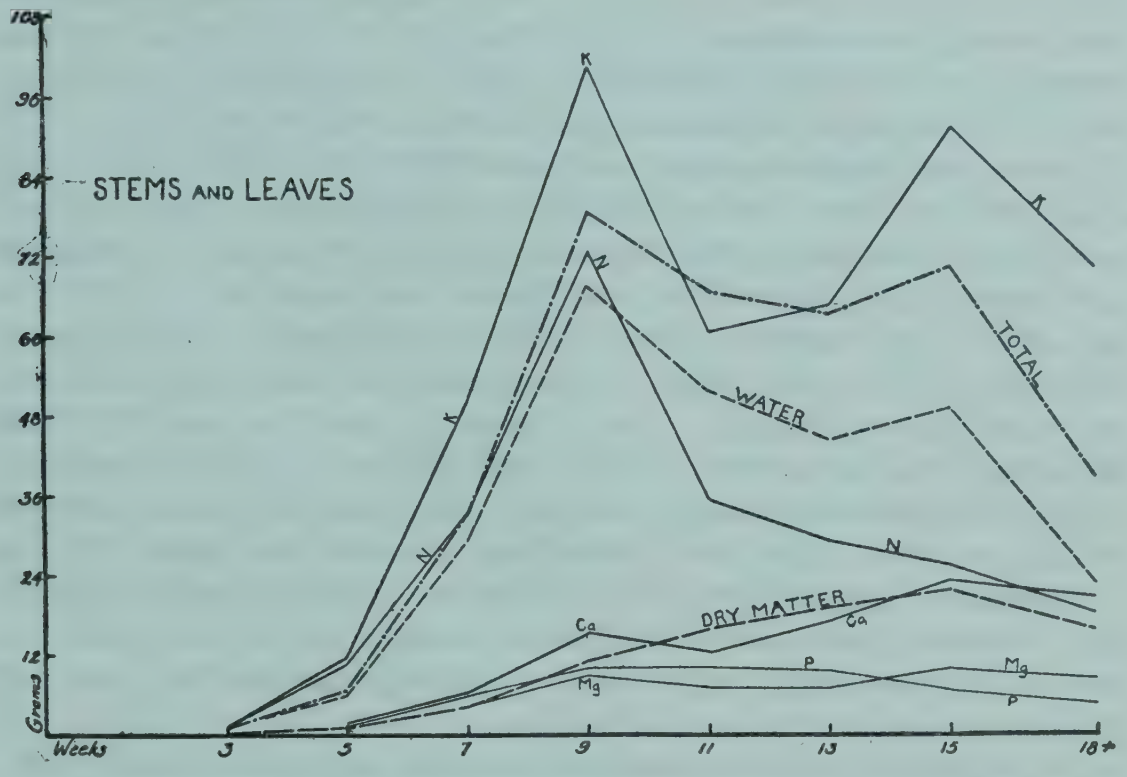

FIG. 7.—Relation of growth of barley to absorption of potassium, nitrogen, phosphate, calcium, and magnesium, for stems and leaves. To emphasize the comparative differences in absorption of these elements, the quantities actually found have been multiplied by 250. Experiment of 1917.

from rain at all times. If we eliminate the rather remote possibility of losses of volatile nitrogen from the leaves, which seems all the more improbable since potassium could not be eliminated in that way, it would seem that the constituents lost either became localized in the roots or returned to the soil. Either condition would represent a very important phenomenon; but migration of potassium and nitrogen from the plant into the soil is such a complete reversal of the ordinary condition that it must be in obedience to causative changes of considerable magnitude, which may be capable of measurement and if so would presumably shed light on the nutritional relations of plants and soils.

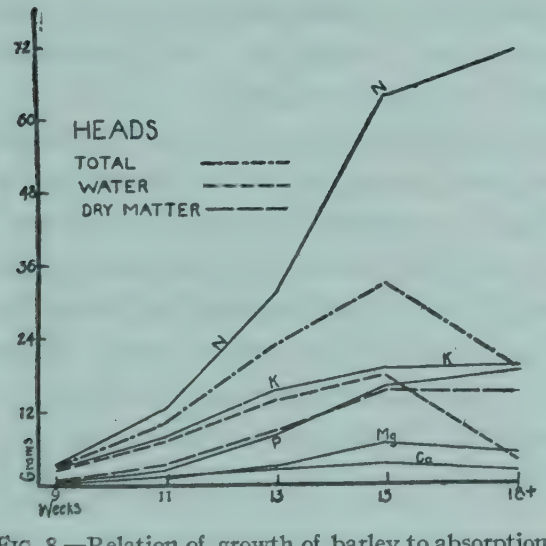

FIG. 8.—Relation of growth of barley to absorption of potassium, nitrogen, phosphate, calcium, and magnesium, for heads. To emphasize the comparative differences in absorption of these elements, the quantities actually found have been multiplied by 250. Experiments of 1917.

The data we have presented above are conclusive only of losses of nitrogen and potassium from the part of the plant growing above ground, inasmuch as we did not include the roots in our study.

We have explained heretofore our reasons for omitting a study of the roots of plants growing in natural soils. For the same reasons we have little confidence in the exactness of the findings of other investigators from similar experiments. It is interesting to note, however, that computations from the well-known work of Wilfarth, Römer, and Wimmer (*9*) show that of the elements in question not more than 10 per cent of the total nitrogen absorbed was contained in the roots at any stage of growth, and only 3 per cent of the total potassium. Data from the same authors, including the roots and stubble with stems to a height of 5 cm. above the surface of the soil, show a maximum of 22 per cent of the total nitrogen and 18 per cent of the total potassium at what appears to be about the same stage of development as our plants when the observed losses took place. Data obtained in this laboratory (*3*) from the same strain of barley grown in sand cultures at a little later stage of growth showed the roots to contain 9.6 per cent of the total nitrogen and 7.3 per cent of the total potassium content of the plant. In our experiment in 1917 the proportions of the total nitrogen and potassium lost from the upper part of the plant, including all of the stems and most of the stubble (see method of cutting described on page 53), were 38 and 34 per cent, respectively.

The magnitude of these losses as compared with the amounts of the elements found in the roots in the cases cited seems sufficiently great to justify the opinion that there is an actual movement of potassium and nitrogen from the plant into the soil at this stage of development. The losses, it will be observed, occur at the time the heads are beginning to form and to draw upon the remainder of the plant for these same constituents (fig. 5–8). There is evidently a concurrent migration of important constituents from the stems and leaves in two directions into the heads and into or through the roots into the soil.

The losses to which we have called attention are not to be confounded with those which apparently take place in numerous plants at the extreme end of the growing season. Here the losses occurred comparatively early in the growth cycle of the plant and were by no means final, being followed by appreciable gains in 1916 and by very substantial increases in 1917. However, the other kind of loss is also to be noted here, there being appreciable losses of potassium, calcium, and magnesium after the fifteenth week when the grain was ripening, in the 1917 experiment, and some indications of the same sort of thing in the work of the preceding year.

The experiments reported by Wilfarth, Römer, and Wimmer (*9*) show evidence of similar losses of potassium and nitrogen and also sodium, at what appears to be about the same early stage [1] of development of

[1] An exact comparison of the two studies is not possible because of differences of soil and climate. In the work quoted, the intervals between cuttings were so much greater (about four weeks as against two weeks) that a closer analogy may have been obscured.

both barley and wheat. The losses noted by them were, however, in every instance final and not succeeded by a further period of absorption as in our experiments.

The behavior of maize as reported by Hornberger (*4*) presents some very interesting analogies to the facts brought out in the present work. It is true that the author quoted shows no absolute losses of constituents derived from the soil prior to the ripening stage. He does show, however, that immediately after the period of maximum absorption and when the heads are beginning to form there is an abrupt slowing down of the rate of absorption of practically all elements. This again is followed by a period of rapid absorption, which in turn is succeeded by the ripening period in which there are absolute losses of all constituents except phosphorus.

Jones and Huston (*5*), also working with maize, showed a period of very rapid absorption of potassium at the eighth week from germination, just prior to the beginning of head formation, followed by a long period of slow absorption, succeeded in turn by a rapid absorption during the sixteenth week, and finally by an absolute loss in the seventeenth. The peak absorptions for nitrogen in the same plants were during the eighth and sixteenth weeks, with an intermediate period of decreased absorption.

On the contrary, the field experiments of Wilfarth, Römer, and Wimmer (*9*) show no losses of potassium and nitrogen at any stage in the growth of potatoes nor very striking changes in the rate of absorption of these elements.

The behavior of maize and barley as contrasted with that of potatoes would seem to indicate that the tendency toward a materially delayed rate of absorption, or absolute loss of constituents at an early stage of development, is probably a characteristic of types of plants whose growth cycle includes a period of extreme differentiation in constructive metabolism. We may, for instance, expect to find such a tendency in other cereal crops and in fruit trees at the period of fruit formation. On the other hand, the discrepancies in the behavior of barley, as shown by important differences of our results from those of Wilfarth, Römer, and Wimmer, indicate that other factors also have an important influence.

SOIL RELATIONS

The abrupt change in the water content and the loss of certain soil constituents from the barley plant at the height of the growing season have no very obvious relation to concurrent changes in external conditions. We can hardly conceive, however, that changes of this kind and magnitude are conditioned entirely by the specific peculiarities of the plants in which they are observed, even though they are not to be found in other types. It is pertinent to inquire more particularly, therefore, into the condition of the soil at the time these important changes take

place. For this purpose we present the data on the periodic water extractions (1 part of soil to 5 parts of water by weight) of the soil of the 1917 experiment.

TABLE V.—*Water extractions of fine sandy loam soil (15)*

[Expressed as parts per million of soil]

Date.	Weeks from planting crop.	Total solutes.		Potassium (K).		Calcium (Ca).		Magnesium (Mg).		Nitrate (NO_3).		Phosphate (PO_4).	
		Cropped.	Uncropped.	Cropped.	Uncropped.	Cropped.	Uncropped.	Cropped.	Uncropped.	Cropped.	Uncropped.	Cropped.	Uncropped.
Apr. 30	0	246	273	22.9	22.6	35.2	40.6	4.1	4.6	53.3	54.5	7.6	7.0
May 14	2	336	371	30.1	27.7	38.3	38.3	8.3	8.2	69.6	67.7	4.7	5.9
21	3	320	353	27.9	30.0	29.6	32.3	20.8	22.3	65.2	63.5	7.1	5.3
June 4	5	247	292	25.3	25.7	23.5	38.0	9.4	11.7	56.4	61.9	5.9	5.8
18	7	178	290	21.2	24.4	17.2	29.0	6.9	13.3	5.7	60.3	4.6	5.8
July 2	9	131	268	14.3	25.1	13.1	25.7	1.7	4.7	8.6	71.1	5.1	7.6
16	11	172	261	15.4	19.0	13.7	19.6	1.8	4.7	2.4	45.7	3.0	4.7
30	13	151	244	22.7	27.4	18.0	30.0	1.2	3.6	4.7	39.9	4.1	6.0
Aug. 13	15	134	224	26.8	31.8	15.7	20.6	2.2	4.1	2.8	37.1	7.8	10.6
27	17	138	277	21.9	33.0	20.2	20.6	2.9	7.1	5.2	77.7	2.3	6.5
Oct. 22	25	222	265	32.8	31.1	30.6	40.3	7.8	12.1	35.0	64.5	8.3	8.1

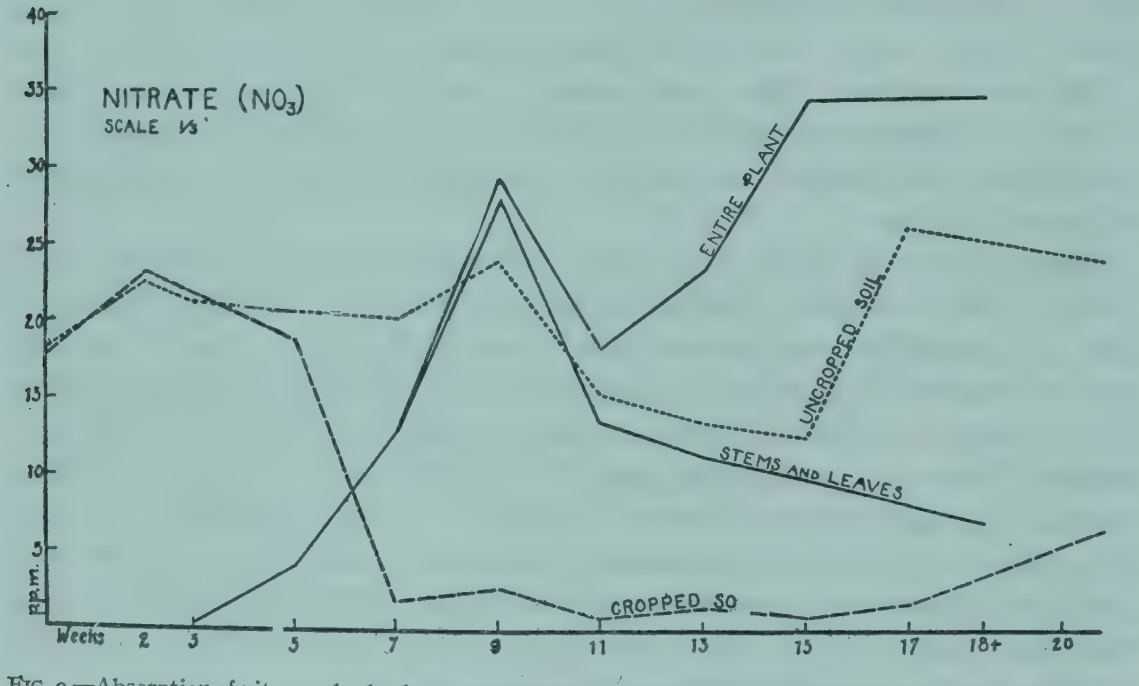

FIG. 9.—Absorption of nitrogen by barley, expressed as the nitrate (NO_3) equivalent and computed to parts per million of soil.

In order to bring out more clearly certain apparent relations between the water extracts and the amounts of soil solutes taken up by the plant, we have computed the plant constituents in terms of the corresponding ions of the soil and expressed these in terms of parts per million of the mass of soil upon which the plants were grown and in which the various constituents must have originated. The data from the soils are expressed in similar terms (fig. 9–13). It should be pointed out at once that the soil data are not to be too literally interpreted. For instance, it is clear that we can not expect that the gains and losses of a given con-

stituent by the plant will be accompanied by exactly equal losses or gains in the soil extract. The more obvious reasons for this are that the water extract is to be regarded only as an indicator of the general magnitudes of

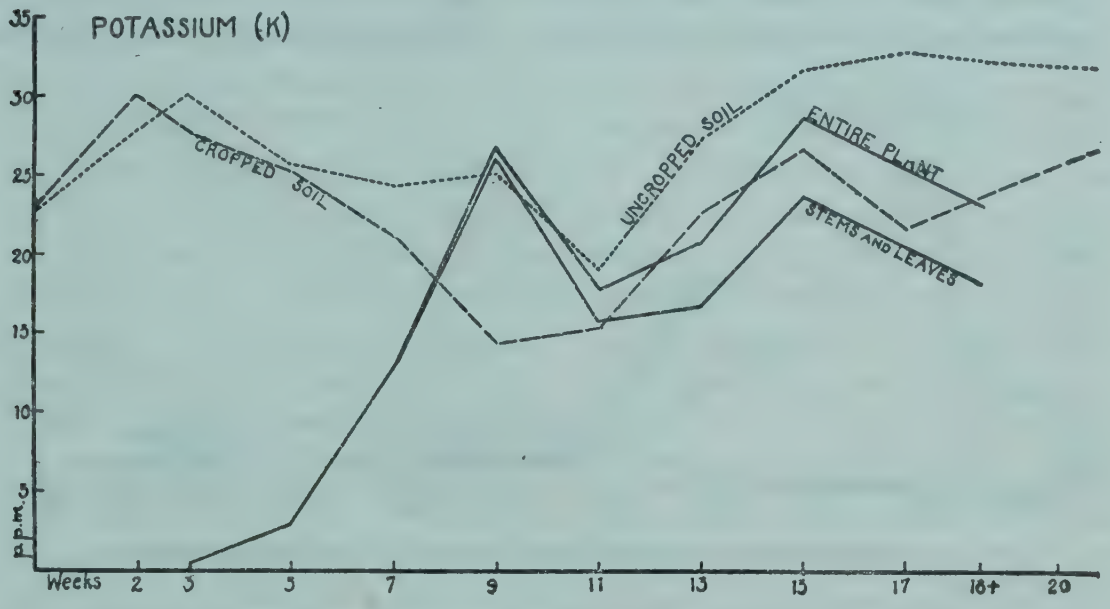

FIG. 10.—Absorption of potassium by barley, computed to parts per million of soil.

the solutes present and not as the equivalent of the soil solution, that ions absorbed by the plants may be partially or entirely replaced in the water extract by solution from the soil minerals, and that solutes lost from the plants may not reappear in the form determined in the soil extract.

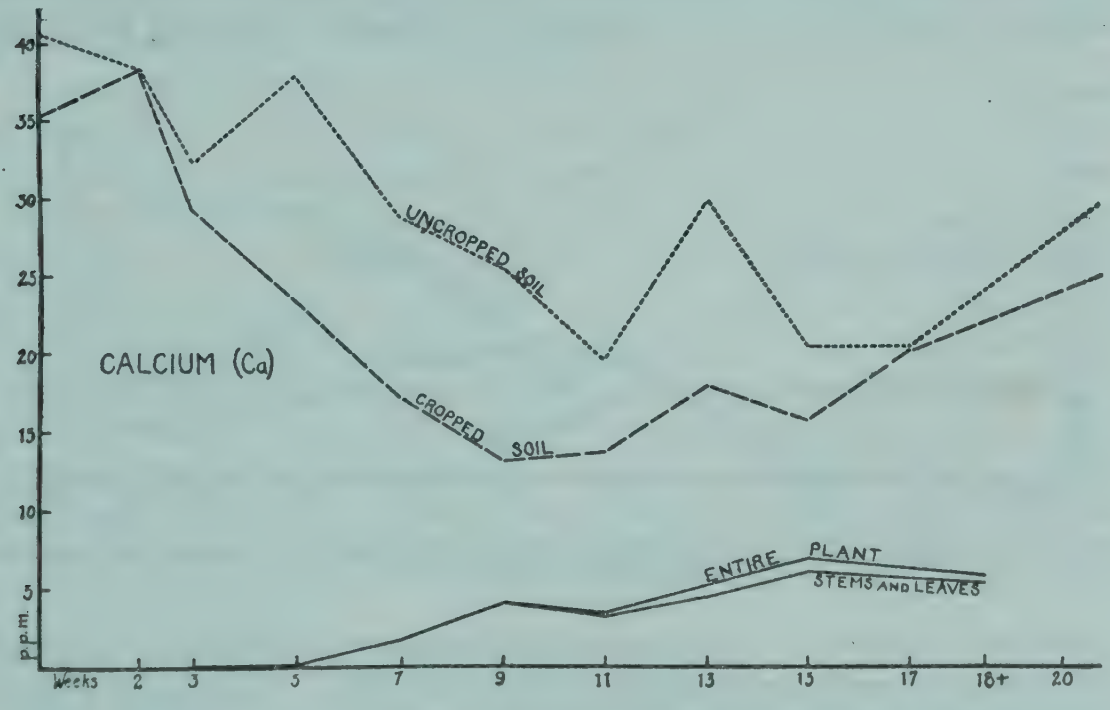

FIG. 11.—Absorption of calcium by barley, computed to parts per million of soil.

NITROGEN ABSORPTION.—(Fig. 9.) The maximum absorption of nitrogen took place between the third week from planting (time of germination) and the ninth week from planting. Almost the entire amount of

nitrogen absorbed could have been supplied by the nitrate in the soil at germination. The additional quantity necessary was presumably supplied by nitrification.

The nitrates in the soil approached a very low level at seven weeks and remained low for the rest of the season. We have repeatedly found such a

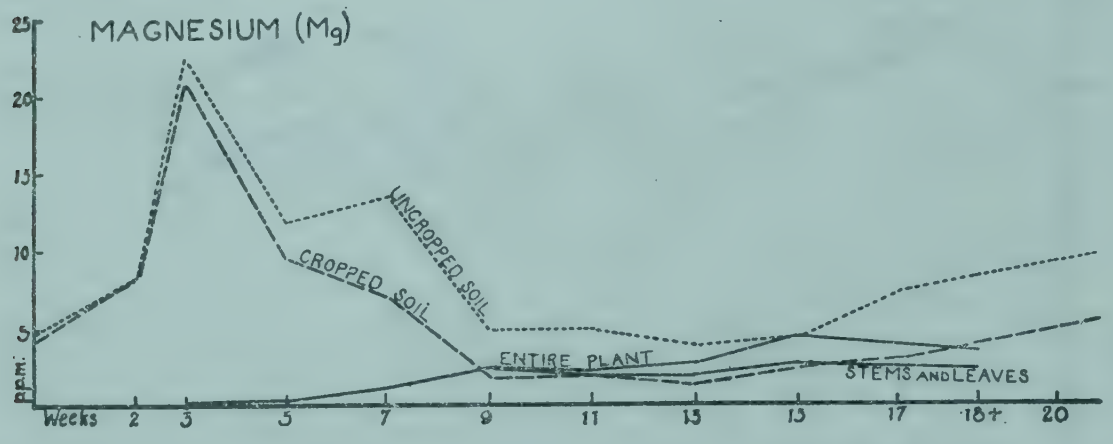

FIG. 12.—Absorption of magnesium by barley, computed to parts per million of soil.

drop to take place in numerous other cropped soils a few weeks after planting. The greatest rate of absorption by the plant occurred between the seventh and ninth weeks.[1] During the succeeding period, ninth to eleventh weeks, the loss of nitrogen from the plant took place. The conjunction of a low nitrate concentration in the soil and high nitrogen content of the plant,[2] soon followed by a movement of nitrogen from the plant toward the soil, is difficult to dissociate, although the exact

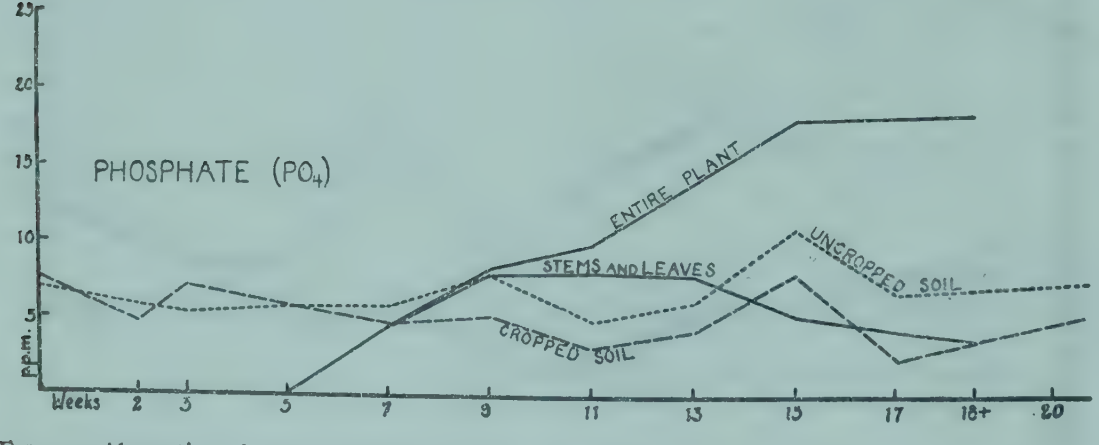

FIG. 13.—Absorption of phosphorus by barley, expressed as the phosphate (PO_4) equivalent and computed to parts per million of soil.

relation is not clear. A further and considerable absorption of nitrogen took place between the eleventh and fifteenth weeks. The rate of absorption, however, was never again so great as that of the period between the

[1] Nitrates, as such, were found in the plants in decreasing quantities up to nine weeks from planting.

[2] Determinations of the various forms of soluble nitrogen in the fresh plant substance at this stage would doubtless give interesting data in connection with the point under discussion here but were not feasible under the conditions of these experiments.

seventh and ninth weeks, and in spite of a continued low nitrate concentration we observe no further loss of nitrogen from the plant. The small additional increment of nitrogen subsequently absorbed, above that present at nine weeks, may be accounted for by concurrent nitrification during the 6-week period between the ninth and fifteenth weeks. The fact that the uncropped soil shows a loss of nitrate at this time is no evidence against such an assumption, inasmuch as nitrification may have been more intense in the cropped soil of low nitrate concentration.

POTASSIUM.—(Fig. 10.) As with nitrate, we observe here a lowered concentration of the soil extract coincident with a high rate of absorption by the plant and shortly afterward followed by a loss of the element from the plant. A little later potassium is again taken up by the plant at a period when the water-extractable potassium of the soil is increasing. If a loss of water-extractable potassium indicates a lowered concentration of the soil solution, some disturbance of the equilibrium between the latter and the cell sap must occur. Increased concentrations of potassium compounds with diosmotic properties in the cell sap would tend still further to change the previously existing equilibria and, if sufficiently great, account for a movement of potassium from the plant to the soil.

CALCIUM AND MAGNESIUM.—(Figs. 11, 12.) The small magnitudes involved in the fluctuations of these elements within the plant vitiate any definite conclusions therefrom. It is interesting to note, however, that there is a distinct loss of calcium and an apparent loss of magnesium between the ninth and eleventh weeks, the period immediately following a lowered concentration of the corresponding ions in the water extracts.

PHOSPHORUS.—(Fig. 13.) The water-soluble phosphorus of the soil seldom shows the considerable fluctuations observed in other elements concurrently with changes in the depression of the freezing point or with changes of soil conditions suggestive of corresponding changes in the soil solution. We can, therefore, hardly expect that the water-extractable phosphorus will shed much light on the mechanism of the process by which plants gain or lose very small increments of this constituent. We may, however, point out that the data have the minor merit of not being inconsistent with the suggestion made in connection with other constituents, in that phosphorus is the only element which appears to increase between the ninth and eleventh weeks and the only one in which the corresponding ion of the water extract does not decrease materially just before this period.

CONCLUSIONS

The absorption of certain soil constituents by barley is characterized by three distinct phases, coextensive with the more important stages of vegetative development. The first of these covers a period of progressively increasing rate of absorption, ending about the time the heads begin to form. At this time the absolute amounts of potassium and nitrogen

contained in the plant approach the magnitudes present at complete maturity. The potassium content may even be greater than at maturity. The beginning of the second phase is indicated not merely by a decreased rate of absorption as in maize but by definite and substantial losses of certain consitutients (notably potassium and nitrogen and apparently calcium) from the portion of the plant growing above the ground, and presumably from the entire plant. This loss takes place concurrently with the migration of the same constituents into the developing heads. The end of the second phase is characterized by a tendency to absorb again the soil constituents previously lost. This tendency may result in taking up considerable quantities when the plants are very large and well developed, as in the 1917 experiment. The third phase, occurring at the time of ripening of the grain, is marked by a practically complete cessation of absorption of all constituents and an actual loss of most of these.

The more significant facts brought out here would appear to be: That the two elements with which plant growth in general is most closely associated may approach or exceed their maxima at a comparatively early stage of the plant's development—that is, at the beginning of head formation; that absorption of potassium and nitrogen during the first period of growth is approximately proportional to the growth attained, and in the succeeding periods the final dry-matter content of the crop more than doubles without any very substantial increase in nitrogen content and with an actual loss of potassium; furthermore, that the final dry-matter content of the crop, even when it varies as much in yield as in the cases reported, appears to be nearly proportional to the fresh weight of the crop at the end of the first period. A direct relation is thus traced between the amount of dry matter in the final yield and the amounts of potassium and nitrogen absorbed in the first stage.

The fact that nitrogen and potassium tend to leave the plant just after the heads begin to form does not prove that their presence is inimical to head formation (no actual losses occur in maize, for example), but indicates rather that continued absorption at this stage is probably incompatible with normal development.

The explanation of the mechanism by which losses of certain constituents take place from growing plants at an early stage of growth must await further detailed studies and a considerable advance in our knowledge of plant metabolism and particularly of the forms in which the various elements exist in the cell sap during the period of translocation to the heads. We shall content ourselves here with the suggestion that such losses are probably due to complex but purely physical causes, a suggestion rendered plausible by the simultaneous occurrence of low concentrations of the water extracts and the movement of mobile constituents from the leaves to the heads.

The concentration of the water extracts of normal soils under crop and producing good yields varies greatly during the growing season (*8*). With barley and numerous other plants such concentrations may become relatively low after the plant becomes well established. Nitrates in particular tend toward a very low level in cropped soils at the height of the growing season even if present in considerable quantities at the time of planting the crop. Since the variation of the freezing-point depressions appears to accord closely with the water extracts (*2*), it seems clear that the normal habitat of annual land plants includes a soil solution which may be of relatively high concentration at the beginning of the growing season but which inevitably falls off at a certain stage of development if the growth is at all prolific. In the light of the results reported above, this diminution of total and nitrate concentration, which we believe to be general in cropped soils, doubtless has an important and probably a favorable effect on crop production. This is confirmed with respect to nitrogen by the abundant evidence we have that applications of nitrates late in the season delay the maturation of grain crops. We interpret these facts to mean that the mutual relations of soils and plants are such that it is generally desirable to have the large amounts of solutes incidental to relatively high concentrations in the soil solution at the commencement of the plant's growth cycle but that it is unnecessary and may be undesirable to maintain this condition during certain later stages of growth. This conclusion has been successfully utilized in this laboratory (*3*) in formulating water-culture experiments with barley, but may not apply to all crops.

It would seem that studies of the absorption of other plants grown on natural soils, particularly those yielding good crops, have important applications in investigations for determining the conditions for optimum growth by means of sand and water cultures; for while the amount of a given constituent absorbed does not necessarily indicate the quantity essential to proper development, fluctuations in the rate of absorption may be expected to reflect the nutritional peculiarities of the crop and serve as a guide in regulating the concentrations and amounts of solutes at successive stages of growth.

SUMMARY

(1) The composition of barley grown on two different soils was studied at successive stages of growth.

(2) In spite of differences in the character of the soils and the yields obtained, a striking similarity was observed in the growth cycles and in the successive changes in the rates of absorption of the plants.

(3) Attention is called to remarkable losses of potassium and nitrogen from the plant at an early stage of development, which are succeeded by renewed absorption at a later period.

(4) The losses observed occurred when the constituents of the water extracts of the soil were at or approaching their minima and when these same constituents were moving from the leaves to the heads.

(5) The suggestion is offered that for many plants high concentrations of the soil solution at certain stages of growth are probably not necessary and may be undesirable.

(6) A plea is made for further studies of other types of plant by the methods used herein, to obtain data for the more rational formulation of experiments with sand and water cultures.

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RELATION OF THE CONCENTRATION AND REACTION OF THE NUTRIENT MEDIUM TO THE GROWTH AND ABSORPTION OF THE PLANT

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INTRODUCTION

The investigation of the growth of plants from the standpoint of the agricultural chemist involves the study of both the soil and the plant. Until recently far greater attention has been given to the chemistry of the soil than to the nutrition of the plant, yet it is obvious that any satisfactory understanding of crop production is concerned as indispensably with the metabolism of the plant as with the chemical reactions of the soil. It is essential to learn what the plant absorbs and metabolizes as well as what the soil solution contains. Of fundamental importance to either phase of the problem is the recognition of the dynamic nature of both the plant and soil systems. Nearly all the studies of the past in soil chemistry have been concerned with the soil as a static system, and thus we have recorded countless experiments dealing with the total composition, hydrochloric acid extracts, lime requirements, and other gross characteristics of the soil. C. B. Lipman (*23*)[2] has pointed out the great service performed by Cameron in introducing certain physico-chemical considerations into soil studies, even though the neglect of some of the modifying factors led to conclusions at variance with the true state of affairs.

Recent researches recognize the paramount importance of the soil solution as the medium from which plants derive their inorganic nutriment. As a result various attempts have been made to obtain some definite conception of the concentration and composition of the actual soil solution by such methods as those proposed by Morgan (*31*) and C. B. Lipman (*22*). A very great advance in this direction has been made by Bouyoucos and McCool (*3*) in their application of the freezing-point method to the study of soil phenomena.

About four years ago this laboratory became engaged in an extensive research on the water extracts of soils held under conditions of exceptional control (*42*). The freezing-point method just mentioned was used also in determining actual osmotic pressures in the soil solution (*17*). The results from both methods of investigation, reinforced by many

[1] With the cooperation of F. W. Weitz. Acknowledgment is made also of the careful analytical work performed by J. C. Martin and A. W. Christie of this laboratory.

[2] Reference is made by number (italic) to literature cited, p. 114-117.

supplementary studies later, have shown very clearly that the soil solution is never in a state of final equilibrium but on the contrary fluctuates daily and seasonally and is profoundly modified as a result of absorption by the plant in such manner that during certain periods the concentration of the soil solution may be reduced to a very low level. Further work by Bouyoucos and McCool (*4*) recently has confirmed these absolutely essential principles.

Along with the concentration and composition of the soil solution, the reaction, or hydrogen-ion concentration, is a definite chemical factor, which under certain circumstances may become of importance through its modifying influence on the soil solution and on absorption by the plant. Previous work by Gillespie (*13*) and by Sharp and the author (*38*) has demonstrated the common existence of soils of distinctly acid reaction as shown by hydrogen electrode measurements. More recent studies by Gillespie and Hurst (*14*), Plummer (*33*), and Gainey (*11*) indicate that various important deductions relative to the soil solution may be drawn from the data obtained by these methods of investigations.

It has been deemed essential to present the foregoing introduction, since the experiments to be described in this paper have their basis in the theoretical considerations, experimental data, and methods resulting from the researches mentioned above, or others concerned with similar underlying principles. However small the beginning, it is hoped that the present studies in plant nutrition may have a special interest due to the use of the more recent methods of investigation and to the attempt made to correlate the results with such knowledge as we now have regarding the soil solution.

GENERAL METHODS OF EXPERIMENTATION

Since it is impossible to govern the exact concentration, reaction, and composition of the soil solution, any rigid control of nutrient solutions requires the use of water and sand cultures. These methods have been employed since the beginnings of the scientific study of agriculture, yet it is only recently that any systematic attempt has been made to elucidate by their use the fundamental problems of plant nutrition aside from the determination of the elements essential for growth long since established in the literature of plant physiology. Even at the present time the control of conditions is very incomplete. Somewhat surprising is the general absence of chemical control as exercised in the analyses of nutrient solutions or of the plants produced. The actual absorption under varying conditions has seldom been studied on any sufficient scale with plants grown for an extended period, although there may be a few exceptions to this statement, as in the recent work by Waynick (*52*) on antagonism and of Schreiner and Skinner (*37*), who have made a large number of analyses of solutions in which wheat seedlings had grown. The question

of absorption, as will be made evident later, involves the whole technic of solution-culture experimentation in its relation to the size of culture vessels and frequency of renewal of solutions, while the interpretation of the data likewise must take into consideration the nature and extent of absorption.

In all of the present experiments a selected Beldi variety of barley was used as the test plant. Although the conclusions of this article are based on experiments with barley, it is our opinion that the general principles of nutrition and absorption deduced apply as well to at least most of the common plants of agricultural interest. Germination was accomplished very conveniently by Waynick's (*52*) method. The bottles for the solution cultures were coated on the outside with black paint and then wrapped with white glazed paper. The latter precaution is very necessary when the cultures are exposed to strong sunlight, on account of heat absorption. Wedges, slightly truncated, were cut from the cork stoppers, and the seedlings were fixed in the openings by means of cotton. As the plants grew and tillered, the size of the openings was increased from time to time by cutting off further segments from the wedge. This part of the technic is important, since there should be no mechanical restriction to the development of the plant. In fact it may be stated as a general principle that the technic employed should place no limitation on the growth of the plant other than that caused by the variables under consideration.

Two sizes of bottles were used, of approximately 1,000- and 2,200-cc. capacity. In some experiments only one plant was placed in each bottle, in others two plants. Thus a relatively large volume of solution per plant was provided. The more detailed discussion of this point is reserved until later in the article. The volume of solution was maintained as constant as possible by the addition of distilled water daily or sometimes oftener.

In order to support the growing plants, glass rods were fixed in the corks and provided with loose loops made of cotton string. Great care was taken that there should be no crowding together of the leaves, for it is essential that each leaf receive the maximum light, and this would be impossible if the plants were bound tightly to the supporting rod. Most of the corks were dipped in melted paraffin previous to use. Any excess of paraffin is, however, to be avoided, since it may soften in the sunlight and injure the seedling at the point of contact.

In making up the nutrient solution the ordinary supply of distilled water was used. This had been in contact only with glass or block tin. At no time was there any evidence of toxicity due to the distilled water. Comparative tests, using water treated with "G Elf" carbon black as recommended by the Bureau of Soils, did not indicate any advantage in this treatment. Baker's analyzed chemicals were usually employed in making the nutrient solutions. Strong stock solutions of calcium nitrate

($Ca(NO_3)_2$), potassium nitrate (KNO_3), magnesium sulphate ($MgSO_4$), and potassium phosphate (KH_2PO_4 and K_2HPO_4) were made and diluted to the desired degree. The composition of the nutrient solutions was then determined by analysis.

In most investigations on plant nutrition, the question of the iron content of the nutrient solution has not received sufficient consideration. Gile and Carrero (*12*) have very thoroughly examined this matter and have reached the conclusion that in the absence of special precautions, it is often possible that the plant may be inhibited in its growth by an insufficient supply of available iron. Our experience is entirely in accord with this view. The presence of sufficient dissolved iron in the culture solution will depend upon the form and quantity of the iron salt used, upon the concentration and reaction of the solution, and upon the time of standing. By direct qualitative tests with potassium sulphocyanid (KCNS) it is easy to show that in certain cases no dissolved iron is found after a comparatively short time, even though considerable quantities of iron chlorid ($FeCl_3$), iron sulphate ($FeSO_4$), etc. had been added to the solution. The solubility is of course greater in solutions of higher hydrogen-ion concentration; but even in acid solutions, when the total concentration of phosphate (PO_4) is high, all the iron may be precipitated. Iron citrate and tartrate seem to be the most effective forms of iron to use. It is also desirable to add the iron solution or suspension to each culture bottle at the time the solution is changed. In any case there must be assurance that iron is not a limiting factor, as it might easily become in solutions of higher concentrations even when the more dilute solutions were plentifully supplied. The experience of Gile and Carrero (*12*) and actual tests with KCNS on the filtered solution would seem to make possible the necessary control.

METHODS USED IN SAND-CULTURE EXPERIMENTS

The method of sand culture has been extensively employed because such a medium obviously affords a more natural habitat for the plant roots than solution cultures can furnish. The selection of the sand is not a matter of indifference, although in many experiments beach sand has been used. This could scarcely be regarded as an entirely inert substance free of soluble material. For the present experiments "Ottawa" sand was selected. This sand is exceptionally pure. Analysis showed that it contained 99.8 per cent silica (SiO_2). In the latter series of experiments the sand received additional purification by treatment with hydrochloric acid (HCl) and subsequent washing with water. Unless the sand is treated with HCl it is possible that a trace of alkaline-reacting substance may alter the reaction of certain nutrient solutions. After the treatment described above, however, it has not been found that any significant change in reaction takes place as a result of contact with the

sand, at least for limited periods of time. The physical analysis of the sand was as follows:

	Per cent.
Passing 60-mesh sieve	60.0
Passing 80-mesh sieve	26.7
Passing 100-mesh sieve	9.7
Passing 150-mesh sieve	2.1
Passing 200-mesh sieve	.8
Passing finer than 200-mesh sieve	.6

The most valuable method of carrying out sand-culture experiments is that described by McCall (*26*, *28*, *29*). By this procedure it is possible to change the nutrient solution as desired. In our experiments large glazed earthenware jars with a capacity of 5 gallons were used with only four or five plants in each jar. It is possible in this way to grow plants without restriction of root development, which might not be possible in small jars. It was not found convenient to withdraw the solution through tubes placed in the bottom of the jars, so a siphon arrangement was finally adopted. A wide-bore glass tube was placed so that it extended to the bottom of the jar. The upper part was bent at right angles and connected through a receiving bottle to a Nelson suction pump. To prevent the sand from being carried over with the liquid a filter of two thicknesses of muslin cloth was fixed on the lower end of each glass tube and held in place by a tightly fitting rubber tube. The use of a little air pressure served to free the filter from any finely dissolved material which might tend to stop the flow of solution. When such arrangements are properly made, six jars simultaneously can be sucked free of all excess solution in a short period.

Water transpired or evaporated was replaced by the addition of distilled water, making up to original weight. The jars were moved about by means of a traveling pulley. One difficulty connected with the addition of water at the top is its tendency to wash down nutrients to the bottom of the jar so that the concentration of the solution is not uniform. In the final sand-culture experiments this has been largely overcome by the use of a glass percolator of 1-liter capacity inverted in the middle of the jar and not filled with sand. Most of the water was added through the projecting tube of the percolator and became equally distributed throughout the jar. In order to test the concentration of the solution, portions of sand were removed at various intervals and freezing-point lowerings determined.

METHODS OF CONTROL

Various methods of control were used during the investigation. These included determinations of osmotic pressure by the freezing-point method both in solutions and when necessary in the soil or sand directly, described by Bouyoucos and McCool (*3*). For estimating hydrogen-ion con-

centration the colorimetric methods of Clark and Lubs (*8*) were employed, or, for estimating soils, direct measurements by means of the hydrogen electrode, in the manner described by Sharp and the author (*38*). Reactions have been calculated to the customary P_H values. Conductivity measurements were carried out in the usual way at a temperature of 25° C. For standardization of the electrode a *N*/50 potassium chlorid (KCl) solution was used.

Since conductivity measurements express the total conducting power of the solution and are necessarily influenced by the nature of the ions, degree of dissociation, etc., they can be regarded as giving only approximate values, showing the general trend and magnitudes of absorption. For the latter purpose they are very convenient, and with the nutrient solutions and concentrations used in this experiment it has been found that the resistance varies with the concentration in a fairly direct ratio.

In many instances chemical analyses for calcium (Ca), magnesium (Mg), phosphate (PO_4), nitrate (NO_3), sulphate (SO_4), and potassium (K) have been made on the solutions or the plants. Whenever possible standard gravimetric and volumetric methods have been used. Occasionally, when very small concentrations were involved, the special technic described by Stewart (*42*) has been found valuable.

In the study of the absorption by the plant the culture jars were made up to the original weight with distilled water then thoroughly mixed by passing a current of air through, after which samples were taken for conductivity measurements or composites made for analysis. Before the jars were made up to weight the cork and plant were removed and temporarily placed in another jar. The roots were allowed to drain as thoroughly as possible, and since they were equally saturated with liquid at all times it is not probable that the general tendency of the results would be appreciably influenced by the loss of solution adhering to the surface of the roots. In several experiments conductivity measurements have been made on each individual jar in order to ascertain the degree of variability displayed by individual plants.

PRELIMINARY SAND CULTURES, SERIES I

Several years ago an attempt was made by the use of sand cultures to gain some idea of the effect of concentration of the nutrient solution on the growth of the barley plant. At this time arrangements were not available for changing solutions, and additional quantities of nutrient solution were added as water was lost by transpiration. Thus there was no control of exact concentrations as there was in the later experiments. It is thought worth while, however, to give these results a brief consideration, since they clearly indicate a definite relation between the nutrients present and the yield of grain and straw. In the critical discussion of other experiments these earlier data may be very helpful.

In this first series of sand-culture experiments 3-gallon jars were used, with seven plants in each jar. Nutrient solutions were added at the beginning to make a moisture content of about 14 to 15 per cent. Later, as transpiration occurred, more solution was added to each jar to make up for water lost. The total quantity of nutrient solution applied to each jar was approximately 5,000 cc. The composition of the nutrient solution was based on a general formula given in texts on plant physiology and was as follows:

	P. p. m.		P. p. m.
NO_3	163	Mg	34
K	85	Na	68
Ca	43	Cl	105
PO_4	163	SO_4	140

Concentrations were in the proportions of 200, 400, 800, and 1,600 parts per million of total salts. For each concentration 7 jars were used, with a total of 49 plants. The plants were cut when the grain was in the "hard dough" stage, and separation of heads and straw was made. The roots were recovered from the dried sand and freed as far as possible from adhering sand. Analyses were made on composite samples of the dried material, separating the plant into heads, straw, and roots. The determinations on the roots were calculated to a silica-free basis. In the following table are presented the data for yields in terms of dry weights, with percentages and total quantities of various elements.

TABLE I.—*Weight and composition of barley*

SAND CULTURES, SERIES 1

Part of plant analyzed.	Concentration of solution.	Average air-dry weight per plant.[a]	Composition (on water-free basis).					Total quantity absorbed per plant.				
			N.	K.	P.	Ca.	Mg.	N.	K.	P.	Ca.	Mg.
	P. p. m.	*Grams.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Stems and leaves..	200	0.44	0.60	1.49	0.46	0.65	0.42	0.0026	0.0062	0.0019	0.0027	0.0018
	400	.96	.59	1.77	.54	.53	.28	.0055	.0163	.0050	.0049	.0026
	800	2.29	.61	2.18	.58	.50	.29	.0135	.0480	.0128	.0110	.0064
	1,600	5.00	.55	2.38	.57	.42	.25	.0270	.1170	.0280	.0205	.0125
Heads.....	200	.10	1.29	1.23	.45	.19	.20	.0012	.0011	.0004	.0002	.0002
	400	.31	1.24	.90	.44	.14	.23	.0037	.0027	.0013	.0004	.0007
	800	.91	1.41	.82	.42	.10	.18	.0123	.0071	.0036	.0009	.0015
	1,600	2.68	1.49	.94	.52	.07	.15	.0372	.0234	.0129	.0018	.0038
Roots[b]	200	.15	.65	1.47	.46	.19	.17	.0010	.0017	.0007	.0003	.0002
	400	.27	.65	1.20	.43	.21	.27	.0017	.0030	.0011	.0005	.0007
	800	.33	.74	1.23	.41	.34	.28	.0024	.0039	.0013	.0011	.0009
	1,600	.87	.80	1.29	.78	1.03	.13	.0068	.0110	.0066	.0087	.0011

[a] Barley grown in adjacent soil tanks at the same time gave from two to five times the yield from best sand culture.

[b] Calculations made on silica-free basis.

After the first few weeks very marked differences were noted in the appearance of the cultures, and with each successively higher concentration the growth was apparently nearly doubled. These general observations were corroborated by the final yields.

In this preliminary experiment the point to be emphasized is the direct relation of nutrients to yield of both grain and straw when either concentration or total supply is insufficient, and also the fact that the total quantities of Ca, K, Mg, PO_4, and N absorbed per plant vary directly with the concentration and total supply of the nutrient solution and in some cases are roughly proportional. The percentages on the basis of dry weight are, on the whole, not very dissimilar. In the heads the higher yielding plants show a lower percentage of some elements on account of the production of a better filled grain. In the straw the percentage of K increases with increasing concentrations.

It is of some interest to compare in each concentration the total quantities of important elements found in the crop with the total quantities added to the sand during the season. These figures are given in Table II.

TABLE II.—*Percentage of absorption of total nutrients added to jars*[a]

SAND CULTURES, SERIES I

Concentration of solution (in p. p. m.).	N.	K.	P.	Ca.	Mg.
200	55	45	24	30	27
400	72	64	35	33	29
800	82	75	36	32	28
1,600	103	96	49	39	27

[a] Calculations made on basis of total quantities contained in whole plants.

It will be observed that somewhat similar percentages of Ca and Mg were absorbed in each of the four different concentrations but that higher percentages of K, PO_4, and NO_3 were absorbed from the higher concentrations. In fact within the limits of error all of the K and NO_3 were utilized by the plant. When these data are later considered from a critical standpoint in connection with questions of supply and concentration, it will become evident that the data for absorption do not represent simply the influence of concentration. They may be interpreted to mean that in the lower concentrations the total supply was insufficient during the first part of the growth period, thus stunting the plant in such a way that later additions of nutrients could not be absorbed at a maximum rate, as they could be in the highest concentration. It should be stated finally that these sand cultures were placed out of doors in good light, adjacent to crops grown in a number of different soils at the same time. In all these soils the crop yields were much superior to those produced in the highest yielding sand culture. Limitations in the nutrient media of the sand cultures must therefore have existed and undoubtedly are to be ascribed to deficient total supply.

SAND CULTURES, SERIES 2

In any experiment similar to the one first described, it is clearly unjustifiable to interpret the results in terms of concentration or ionic ratios. Such an interpretation is warranted only when an opportunity is afforded for controlling the concentration of the solution at all times. In this second experiment the method of McCall (*26*) has been adopted, as previously stated. Solutions were changed during the period of active absorption every three days; and observations on the sand showed that the solutions were kept relatively constant, although on some occasions the leaching of nutrients to the bottom of the jar caused considerable fluctuations.

The plants were grown out of doors from August to December. There was abundant vegetative growth, but the temperature and light conditions did not permit the production of grain. The plants were still green when cut. We may use the data from this experiment, therefore, to indicate the general relation of concentration to yield in terms of dry weight of tops and roots and to the absorption of important elements while the plant is in an active state of metabolism.

The composition of the nutrient solution used in this and subsequent experiments had its basis in the analyses of water extracts of soils which had been under observation for several years, as described by Stewart (*42*). The nutrient solution was so constituted as to give approximately the same relations between the more important ions as that found in the water extracts of fertile soils at the period when the crop was actively absorbing. For the purposes of the present investigation this seemed as logical a basis as any for making up the nutrient solution. Possibly other combinations might give somewhat higher yields at times, but the ratios between the elements in the solutions employed were certainly not unfavorable. Moreover, within wide limits the broad relations of reaction and concentration to the course of absorption will not be affected by small differences in ionic ratios.

The nutrient solution of 0.78 atmospheres osmotic pressure had the following composition:

	P. p. m.
NO_3	700
K	284
PO_4	136
Ca	200
Mg	99
SO_4	368
NaCl	30

P_H=6.8.

The other concentrations used were 0.10, 0.25, 0.48, and 1.45 atmospheres.

The yields and analyses of plants are given in Table III.

TABLE III.—*Weight, composition, and total absorption per plant*

SAND CULTURES, SERIES 2

STEMS AND LEAVES

Concentration of solution.	Average air-dry weight per plant.[a]	Composition (on water-free basis).									
		Total N.	Nitrate N.	Total P.	Water-soluble P.	Insoluble P.	Total K.	Water-soluble K.	Insoluble K.	Total Ca.	Water-soluble Ca.
Atmospheres.	*Grams.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
0.10	6.2	2.65	0.02	0.72	0.62	0.10	3.97	3.44	0.53	1.00	0.85
.25	15.3	3.14	.14	.84	.66	.18	5.38	4.65	.73	1.21	.81
.48	24.0	3.85	.37	.95	.75	.20	6.90	6.10	.80	1.24	.77
.78	25.5	4.03	.46	.92	.77	.15	7.59	7.08	.51	1.09	.73
1.45	26.5	4.01	.52	.85	.71	.14	7.63	6.85	.78	.99	.70
[b]0.10 to .48	13.8	3.87	.51	.91	.67	.24	7.47	6.73	.74	1.27	.81

ROOTS

0.10	1.30	2.35	.....	0.48	.....	.....	.....	.....	.....	0.49	.....
.25	1.85	2.46	.....	.60	0.31	0.29	.....	2.75	.....	.66	0.52
.48	2.95	3.61	0.30	.94	.41	.53	4.43	4.07	0.36	1.34	.60
.78	3.85	3.97	.50	1.45	.44	1.01	6.38	5.62	.76	2.67	.74
1.45	3.80	3.67	.69	2.42	.40	2.02	5.23	5.09	.14	4.42	.82
[b]0.10 to .48	2.10	4.03	.....	.88	.48	.40	.....	4.39	.....	1.03	.94

STEMS AND LEAVES

Concentration of solution.	Average air-dry weight per plant.[a]	Composition—Continued.				Total quantities absorbed.				
		Insoluble Ca.	Total Mg.	Water-soluble Mg.	Insoluble Mg.	N.	P.	K.	Ca.	Mg.
Atmospheres.	*Grams.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
0.10	6.2	0.15	0.40	.....	.....	0.154	0.041	0.230	0.058	0.023
.25	15.3	.40	.43	0.41	0.02	.456	.122	.780	.176	.062
.48	24.0	.47	.44	.39	.05	.878	.216	1.570	.283	.100
.78	25.5	.36	.43	.36	.07	.988	.227	1.860	.267	.105
1.45	26.5	.29	.42	.42	.00	1.020	.215	1.935	.252	.107
[b]0.10 to .48	13.8	.46	.48	.42	.06	.506	.120	.978	.166	.063

ROOTS

0.10	1.30	.....	0.22	.....	.....	0.031	0.006	0.017	0.006	0.003
.25	1.85	0.14	.35	0.23	0.12	.045	.011	.049	.012	.007
.48	2.95	.74	.42	.36	.06	.106	.028	.130	.039	.012
.78	3.85	1.93	.62	.44	.18	.153	.056	.246	.103	.024
1.45	3.80	3.60	.71	.44	.27	.140	.092	.198	.169	.027
[b]0.10 to .48	2.10	.09	.62	.60	.02	.085	.018	.076	.022	.013

[a] Averages of 10 plants except in the 0.10 and 0.10 to 0.48 atmosphere concentrations, which were averages of 5 plants. Mean deviation per plant approximately ± 2 gm., for tops.

[b] Cultures started with solution of 0.10 atmosphere concentration, after 52 days changed to 0.48 atmosphere.

The dry weights indicate that under these conditions a concentration of 0.10 atmospheres was too low, 0.25 atmospheres possibly sub-optimum, while no important difference between 0.48 atmospheres and 1.45 atmospheres was referable to the nutrient solutions. In other words, the optimum concentration—that is, the least concentration giving a maximum yield—would be found between 0.25 atmospheres and 0.48 atmos-

pheres. It is not certain, however, that the 0.10 atmospheres concentration was always constant, in spite of the great volume of solution used.

Especially interesting are the data showing in percentages the composition of the different parts of the plant. Some idea may be gained here of the influence of concentration on the absorption of individual elements and of the distribution between roots and tops. In the first place, the analyses of the tops show an increase of N from 2.65 per cent in the concentration of 0.10 atmospheres to a maximum of 4.03 per cent in the concentration of 0.78 atmospheres. Likewise the total K increases nearly 100 per cent. No such increase is noted, however, for PO_4, Ca, or Mg. The NO_3 also increases regularly with increasing concentration, and in the higher concentration about 0.5 per cent of nitrate nitrogen is found.

The elements soluble in cold water were determined by shaking the dried and ground sample with an excess of water, filtering, and then estimating the total quantity of each element found, the final calculation being based on the weight of dry material. The striking increase in percentage and total quantity of K in the tops of each plant is largely referable to K soluble in water. Most of the P and almost all of the Mg were soluble. Only about two-thirds of the Ca was soluble.

When we examine the data from analyses of the roots we meet with a different set of relations. There is an increasing percentage and total quantity of N and K, as in the tops; but, unlike the tops, the roots show very large increases in PO_4, Ca, and Mg. The partition between soluble and insoluble fractions shows that the increase is principally due to insoluble forms of the elements. The data suggest the hypothesis that with increasing concentration insoluble phosphates of Ca and Mg are precipitated in the roots, while the tops of the plants principally store excess N and K.

It should be repeated that in this experiment the large containers (5-gallon jars), together with frequent changes of solution, maintained, with the possible exception of the lowest concentration, an approximately constant concentration at the different levels. Thus the results indicate, at least in a general way, the influence of concentration rather than the limitation of insufficient total quantities. When such concentrations are maintained continuously, it is apparent that the plant may attain a condition in which the percentage of certain elements in its composition is very high, far higher than for plants grown under different conditions or in the field. It is of course understood in a general way that fertilization may affect the composition of the crop, but only in an experiment of the kind outlined above is it possible to point out definite and logical relationships. Also, it is practically impossible to obtain reliable results from the roots of plants except in sand and water cultures. It is not practicable to recover the roots from the soil quantitatively, and contamination is unavoidable. The results from this experiment are

therefore of interest in themselves and will also be pertinent to the further discussion of the course of absorption by the plant.

During the growth period of this series frequent observations were made on tillering and height. Except in the lowest concentration, heights were practically uniform. The number of tillers per plant, however, seemed to increase with increasing concentration. The total yield of dry matter per plant justifies the conclusion that under the conditions of experimentation here described there is no restriction on the optimum production of vegetative growth traceable to insufficient nutrients or limited size of containers.

SAND CULTURES, SERIES 3

During the following summer a further set of sand-culture experiments was carried out. The technic was similar to that of the preceding experiment except for the improved method of distributing the solution, described in the first part of the article. In this series sixteen 5-gallon jars were used with three concentrations of solution of 0.95, 1.95, and 3 atmospheres, respectively. The concentration of solution in four of the jars was decreased after 6 weeks from a concentration of 0.95 atmospheres to one of 0.15 atmospheres, in four other jars after 9 weeks, and in another set after 12 weeks. In the two highest initial concentrations a decrease was made after 6 weeks to 0.95 atmospheres concentration, and after 9 weeks to 0.15 atmospheres. The object of these changes in the concentration of the solution during the growth of the plant was to imitate certain of the conditions actually existing in the soil solution during the growth of the plant, as noted in the investigations of Burd (*6*), Stewart (*42*), and the author (*17*). These experiments showed very clearly that the soil solution diminished in concentration after the plant had grown 8 to 10 weeks. Nitrates at this period almost disappeared. Nevertheless, even with this exhaustion of the soil solution after 8 to 10 weeks, the plants completed their cycle growth, and in most of the soils the ripened crop (after 16 weeks) was characterized by a high yield of both straw and grain. It must follow, therefore, that such a change in the concentration and composition of the nutrient solution at the particular time in question is in no way unfavorable to crop production.

The data presented in Table IV may be considered in the light of the foregoing discussion. While there is considerable variability present, the results may reasonably be accepted as indicating the general trend.

The average yield from the four jars in which the solution was changed to one of low concentration after 6 weeks was possibly inferior to that of the other cultures; but the jars in which the concentration was reduced after 9 weeks gave an equal yield of grain and nearly equal yield of straw, as compared with the cultures in which the highest concentration was maintained for 12 weeks. The initial concentration of 1.95 atmospheres may have had an inhibitive effect, even though later the solution

was reduced to a more favorable concentration. An initial concentration of 3 atmospheres was decidedly injurious.

TABLE IV.—*Weight, composition, and total absorption per plant*

SAND CULTURES, SERIES 3

Part of plant analyzed.	Concentration of solutions and time of application.	Dry weight per plant.[a]	Composition (on water-free basis).				
			N.	P.	K.	Ca.	Mg.
	Atmospheres.	*Gms.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Stems and leaves.	0.95 for 6 weeks: 0.15 for 10 weeks.....	11.2	1.06	0.27	2.78	1.08	0.38
	.95 for 9 weeks: .15 for 7 weeks.....	13.9	1.49	.36	3.86	.96	.28
	.95 for 12 weeks: .15 for 4 weeks.....	13.4	2.48	.52	4.95	1.07	.33
	1.95 for 6 weeks; .95 to .15 for 10 weeks..	10.5	1.38	.42	4.04	1.20	.29
	3.00 for 6 weeks; .95 to .15 for 10 weeks..	7.8	1.40	.44	3.05	1.19	.33
Heads.	.95 for 6 weeks; .15 for 10 weeks.....	7.8	2.77	.67	.89	.20	.23
	.95 for 9 weeks; .15 for 7 weeks.....	10.6	2.75	.64	.86	.18	.22
	.95 for 12 weeks; .15 for 4 weeks.....	9.9	2.93	.70	1.08	.23	.22
	1.95 for 6 weeks; .95 to 15 for 10 weeks ..	9.1	2.62	.69	.73	.22	.22
	3.00 for 6 weeks; .95 to .15 for 10 weeks..	5.9	2.81	.70	.96	.22	.23
Roots.	.95 for 6 weeks: .15 for 10 weeks.....	3.9	.84	.17	.47	.27	.13
	.95 for 9 weeks; .15 for 7 weeks.....	7.1	.61	.24	.45	.47	.08
	.95 for 12 weeks; .15 for 4 weeks.....	4.0	.97	.12	.77	1.76	.15
	1.95 for 6 weeks: .95 to .15 for 10 weeks..	3.5	.72	.42	.58	.58	.67
	3.00 for 6 weeks: .95 to .15 for 10 weeks..	2.8	.55	.44	.53	.78	.77

Part of plant analyzed.	Concentration of solutions and time of application.	Dry weight per plant.[a]	Total quantities absorbed.				
			N.	P.	K.	Ca.	Mg.
	Atmospheres.	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*
Stems and leaves.	0.95 for 6 weeks: 0.15 for 10 weeks.....	11.2	0.119	0.031	0.311	0.121	0.043
	.95 for 9 weeks; .15 for 7 weeks.....	13.9	.208	.050	.538	.134	.039
	.95 for 12 weeks: .15 for 4 weeks.....	13.4	.332	.070	.662	.143	.044
	1.95 for 6 weeks; .95 to .15 for 10 weeks..	10.5	.145	.045	.424	.126	.031
	3.00 for 6 weeks; .95 to .15 for 10 weeks..	7.8	.109	.034	.237	.093	.026
Heads.	.95 for 6 weeks; .15 for 10 weeks.....	7.8	.216	.052	.070	.016	.018
	.95 for 9 weeks; .15 for 7 weeks.....	10.6	.290	.068	.091	.019	.023
	.95 for 12 weeks: .15 for 4 weeks.....	9.9	.290	.069	.107	.023	.022
	1.95 for 6 weeks; .95 to .15 for 10 weeks..	9.1	.239	.063	.067	.020	.020
	3.00 for 6 weeks; .95 to .15 for 10 weeks..	5.9	.165	.041	.056	.013	.014
Roots.	.95 for 6 weeks: .15 for 10 weeks.....	3.9	.033	.007	.018	.011	.005
	.95 for 9 weeks; .15 for 7 weeks.....	7.1	.044	.017	.032	.034	.006
	.95 for 12 weeks; .15 for 4 weeks.....	4.0	.039	.047	.031	.070	.006
	1.95 for 6 weeks; .95 to .15 for 10 weeks..	3.5	.025	.015	.020	.020	.024
	3.00 for 6 weeks; .95 to .15 for 10 weeks..	2.8	.015	.012	.015	.022	.021

[a] Averages of 16 plants, except in two highest concentrations in which averages are calculated for eight plants. Mean deviation approximately ± 2 gm.

The inference from these results is that an optimum concentration and supply of nutrients must be furnished to the plant for perhaps 8 or 10 weeks. Burd's (7) investigations have already shown that very little absorption from the soil takes place during the first 3 weeks of growth, so it must follow that the most critical period extends over only 6 or 7 weeks. If the nutrient medium is favorable during this period, a very low concentration or small supply may suffice for the remainder of the season, although, as will be proved later, active absorption may continue much longer if suitable solutions are provided. Furthermore, we gain the impression from this experiment that concentrations above 2 atmospheres are too great for the barley plant and that the initial stunting is not entirely overcome by a subsequent change to a more favorable

condition. Other pot experiments with soils uphold this view. When a mixed fertilizer was added to the soil so as to produce, by the freezing-point method, an osmotic pressure similar to that just mentioned, equally striking inhibitive effects were noted.

The data for the total quantities and the percentages in the composition are very definite and confirm the conclusions drawn from series 2. Again we find K and N stored in the tops, while the principal accumulation of Ca and P is in the roots. The heads are only slightly affected in their percentages. Evidently not only concentration but the period during which the concentration is maintained markedly influence the percentage of inorganic elements and the total quantity absorbed per plant. These factors of concentration and period of maintenance of concentration in the soil solution doubtless govern the variations of the plant in its content of inorganic elements under any particular climatic conditions.

WATER CULTURES, SERIES 1

Although the method of sand culture devised by McCall offers very great advantages in control, it is not easily possible to determine the exact composition of the solution at any given time. In order to make an intensive study of the influence of the solution on the plant, and of the plant on the solution, it became necessary to employ the method of solution culture. The technic adopted differed from that in general use in that fewer plants and larger volumes of solution were employed. Observations were made at all stages of growth to maturity. The first experiments were carried out in the greenhouse during the winter months, and the later series were placed out of doors in good light during the spring and summer months.

The first series of experiments was planned to furnish preliminary information with regard to the optimum concentration and the relation of concentration to absorption and transpiration. One-liter bottles were used, with 3 plants in each bottle. Solutions were changed every 3 days after the plants had started to make appreciable growth. After 54 days the plants were cut and the dry weight determined. Four concentrations of solution were tested, and for each concentration there were 8 jars, or 24 plants. The composition of the solution was similar to that given by Shive (*39*) for his best cultures. Table V summarizes the most important results obtained from this experiment.

The data indicate that for these conditions a concentration of 0.10 atmosphere is sub-optimum and one of 2 atmospheres super-optimum. Concentrations of 0.32 and 0.85 atmospheres were equally efficient if we consider the mean deviation in the yields of individual plants. The greatest total transpiration occurred in solution 2. Transpiration per unit of dry weight was decidedly greater in lower concentrations.

TABLE V.—*Effect of concentration on growth and transpiration*

WATER CULTURES, SERIES I

Approximate concentration of solution.	Osmotic pressure.	Number of plants.	Total dry weight of tops.	Average dry weight of tops.	Mean deviation.	Total dry weight of roots.	Transpiration per gram dry weight of tops.
P. p. m.	*Atmos.*		*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
200	0.10	24	12.90	0.54	±0.07	3.4	906
800	.32	24	22.95	.95	± .15	4.0	712
2,500	.85	24	21.65	.90	± .10	4.8	593
6,000	2.07	24	15.50	.65	± .09	3.5	624

A series of sand cultures was carried out to parallel the water cultures; but since there was some indication that the paraffine seal had exercised an inhibitive effect on the growth of the plants, these data are omitted. The evidence obtained, however, led to the conclusion that the transpiration per unit of dry weight was greater in the sand cultures than in the water cultures. This is in accord with the findings of Bouyoucos (*2*).

The absorption studies were made by means of conductivity measurements. In each case comparisons were made under identical conditions between solutions before and after contact with the plant. The general trend of absorption is expressed in a graph. The absorption has been calculated in terms of parts per million of total electrolytes absorbed, based on concentration of the original solution.

The total absorption is evidently greater in the solutions of higher concentration, but in the solution of highest concentration there were two periods when an increase was noted in the concentration of the solution after contact with the plant. It may be inferred that the plant had absorbed in the preceding period such an excess of one or more ions as to cause a temporary reversal of the absorption processes, with a return of ions to the solution. These fluctuations are undoubtedly related in some way to the general light and temperature conditions affecting growth. In later experiments carried on out of doors during the spring and summer the absorption followed a more uniform course. In the greenhouse experiment, light conditions were not favorable to a high yield, and considerable fluctuations in temperature occurred.

In almost all experiments designed to show the relation of the concentration of the solution to absorption and transpiration, the seedlings have been grown from the beginning in the solutions which it was desired to investigate. Any nutritive deficiencies in the solution would thus be reflected in the development of the plant, and the fundamental relations would be obscured. It would seem that the problem might be simplified by growing the plant in a favorable nutrient solution until it had reached a stage of active absorption and then transferring it for a

comparatively short period to any desired solution. A large number of plants may be developed to a uniform stage, and we may then compare different solutions from which absorption is taking place with plants of equal leaf and root development. In brief, the plant is regarded merely as a controlled absorbing system to be investigated.

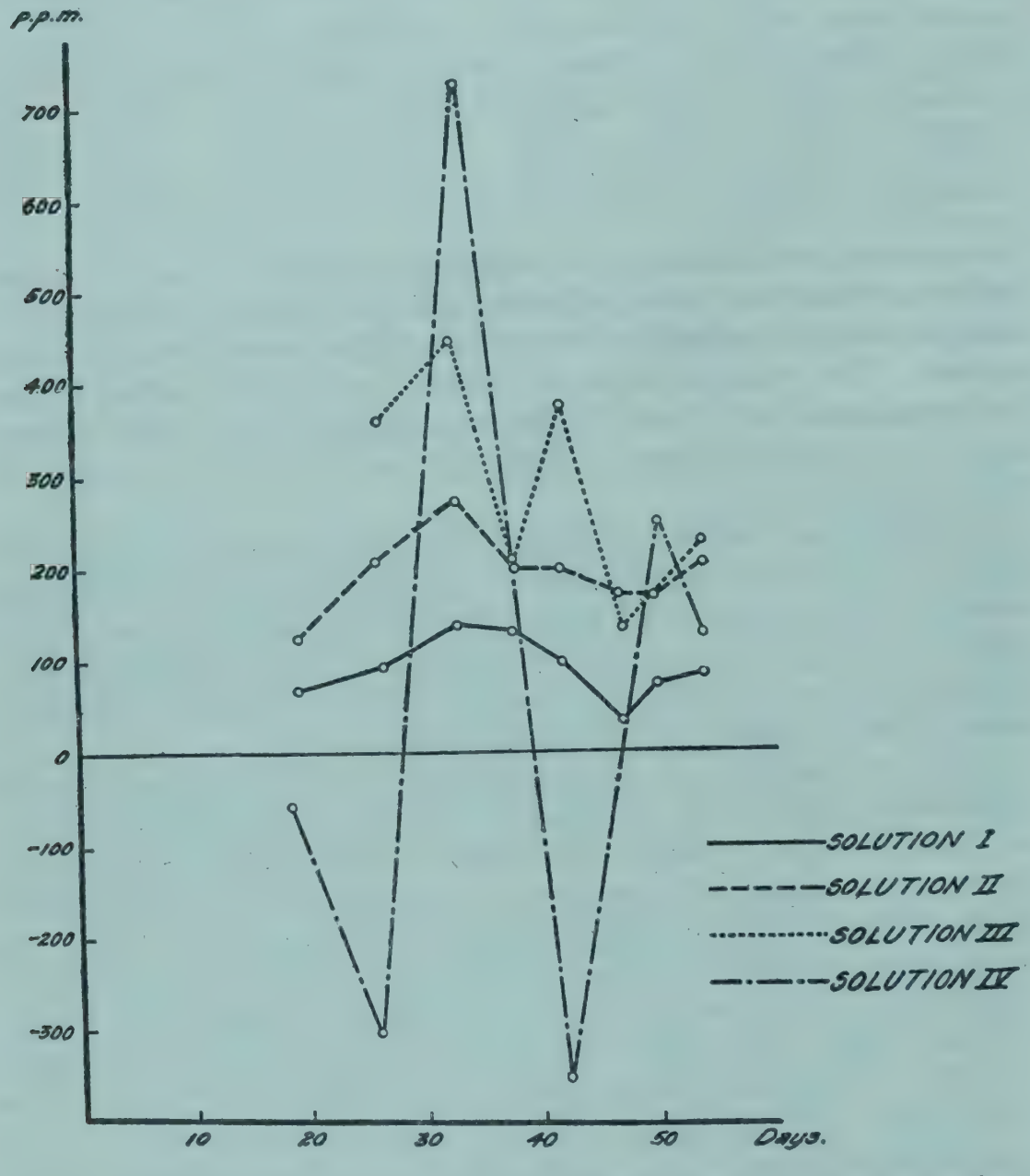

FIG. 1.—Water cultures, series 1. Graph showing net absorption in parts per million from solutions of four concentrations:

Solution I with concentration of 0.10 atmospheres.
Solution II with concentration of 0.32 atmospheres.
Solution III with concentration of 0.85 atmospheres.
Solution IV with concentration of 2.07 atmospheres.

Such a procedure was accordingly carried out in the next experiment. Fifty-six uniform seedlings (2 in each 1-liter bottle) were grown for several weeks in a nutrient solution of 0.85 atmospheres concentration. At the end of this period the bottles were divided into four groups. After the roots were rinsed with distilled water the plants were trans-

ferred to solutions of the four different concentrations previously mentioned. The absorption and transpiration were then determined for a period of two days, after which the plants were cut and the dry weight determined.

TABLE VI.—*Transpiration and absorption by uniform plants*

WATER CULTURES, SERIES I

Approximate concentration of solution.	Osmotic pressure.	Average dry weight of tops.	Transpiration per gram dry weight of tops.		Average absorption of electrolytes from each jar.
			First day.	Second day.	
P. p. m.	*Atmos.*	*Grams.*	*Grams.*	*Grams.*	*P. p. m.*
200	0. 10	0. 43	50	94	−25
800	. 32	. 46	46	91	72
2, 500	. 85	. 50	34	64	288
6, 000	2. 07	. 50	33	48	402

These results are interpreted to mean that the concentration of the solution has a marked effect on absorption and transpiration. Almost double the quantity of water per gram of dry weight is transpired by the plants in the solution of lowest concentration as compared with the quantity transpired by those in the highest concentration. In the solution of lowest concentration there was possibly a slight excretion of electrolytes from the plant. In the other solutions absorption took place in the order of increasing concentrations.

It has sometimes been assumed that transpiration may be regarded as proportional to the dry weight of the plant and that the effect of various solutions may be reflected in the transpiration. Livingston (*24*) and Whitney and Cameron (*55*) have tended toward this view; on the other hand Bouyoucos (*2*) has found that the concentration of the nutrient solution has a decided influence on transpiration, the higher concentrations diminishing water loss, either because of the difficulty experienced by the plant in absorbing water from a solution of higher osmotic pressure or because the increased osmotic pressure of the cell sap decreased the vapor tension and so reduced transpiration. Our experiments uphold the view that solutions of increased concentration have the effect of reducing transpiration. Since plants of uniform development and approximately equal leaf surface display widely different transpiration rates with solutions of different concentrations, it does not appear that transpiration is necessarily an accurate criterion of growth. There is, in fact, a preponderance of evidence to show that transpiration per unit of dry weight increases with decreasing concentration. Kiesselbach (*21*) and Khankhoje (*20*) reached this conclusion as a result of sand-culture studies, while Preul (*35*) and Kiesselbach found that the water requirement per unit of dry weight is less on a poor soil. Widtsoe (*54*) says that fallowing and fertilization decreased the water requirement.

These observations on soils are in harmony with the work of Stewart (*42*) and the author (*17*), who found that the concentration of the soil solution may be increased by fallowing and decreased by cropping.

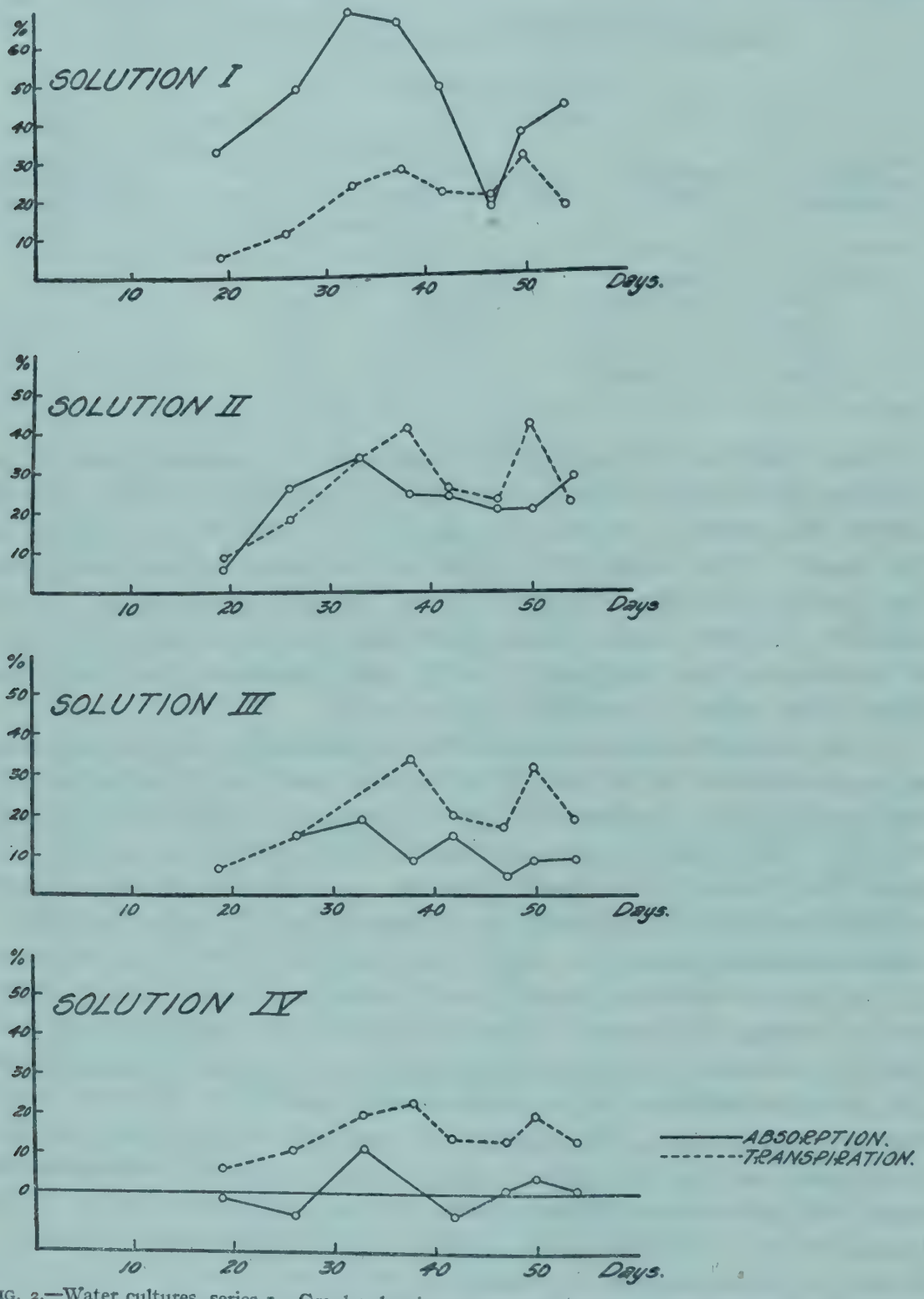

FIG. 2.—Water cultures, series 1. Graphs showing comparison between percentage of total nutrient absorbed and percentage of total water transpired with solutions of four concentrations.

The relation between absorption and transpiration are shown in figure 2. The data have been calculated in terms of percentage of total water transpired and percentage of total nutrients absorbed. Very different

results are given by the different concentrations. In the lowest concentration the percentage of water transpired is much less than the percentage of electrolytes absorbed. In the highest concentration this relation is reversed, and in the intermediate concentration of 0.32 atmospheres the two percentages are essentially the same. It is quite clear that absorption may proceed independently of transpiration, and that the plant may absorb and transpire at such rates as either to increase or decrease the concentration of the nutrient solution. This is in general agreement with the views of Pantanelli (*32*).

WATER CULTURES, SERIES 2

This series was planned for the purpose of making certain observations on absorption and growth when plants were grown to maturity. Two-liter bottles were used, and only one plant was placed in each bottle. The experiment was started on February 11. During the first 10 weeks, up to the time of heading out, a solution of 0.78 atmospheres concentration was used, the solution being changed about once each week. The composition of the solution was similar to that used in sand cultures, series 2. The plants were grown out of doors, and at this season the growth rate was fairly slow. On April 27 the plants were divided into 3 groups of 10 plants each and the solution changed to 3 different concentrations, 0.10, 0.30, and 0.80 atmospheres, respectively. Thereafter the solutions were not changed. The object of this procedure was to gain some insight into the growth and absorption of the plant when the concentration of the solution was diminished during the latter part of the growth cycle, a condition somewhat analogous to that in the soil.

The plants completed the growth cycle and produced matured heads. There were from 5 to 10 tillers on each plant. Most of the heads were out by May 17, and by June 24 the hard dough stage was reached. The plants were cut July 16, about five months after planting. A marked difference in appearance was noted in the three groups. Although water was, of course, not a limiting factor in the concentration of 0.10 atmospheres, the plants turned yellow sooner and more completely than in the higher concentrations. In the highest concentration considerable green color persisted to the end of the experiment, but the heads from the lowest concentration were slightly superior. In the highest concentration the ripening was slow and there was some indication of shrinkage of the grain before the period of desiccation. The total yields as shown below are greater in the higher concentrations, although there is no significant difference between the concentrations of 0.10 and 0.30 or 0.80 atmospheres, for the yield of heads and even the difference in the yields of stems and leaves are not necessarily significant.

AIR-DRY WEIGHT PER PLANT.[a]

Concentration of solution.	Heads.	Stems and leaves.	Roots.
Atmospheres.	*Grams.*	*Grams.*	*Grams*
0. 10	10. 2	10. 6	0. 44
. 30	10. 7	12. 7	. 30
. 80	12. 0	15. 4	. 77

[a] Based on 10 plants.

The conclusion may be drawn from the foregoing observations that the greater concentration or supply of one or more ions present during the later stages prolongs the period of vegetative growth and possibly interferes with the processes of ripening, without producing any large increase in yield.

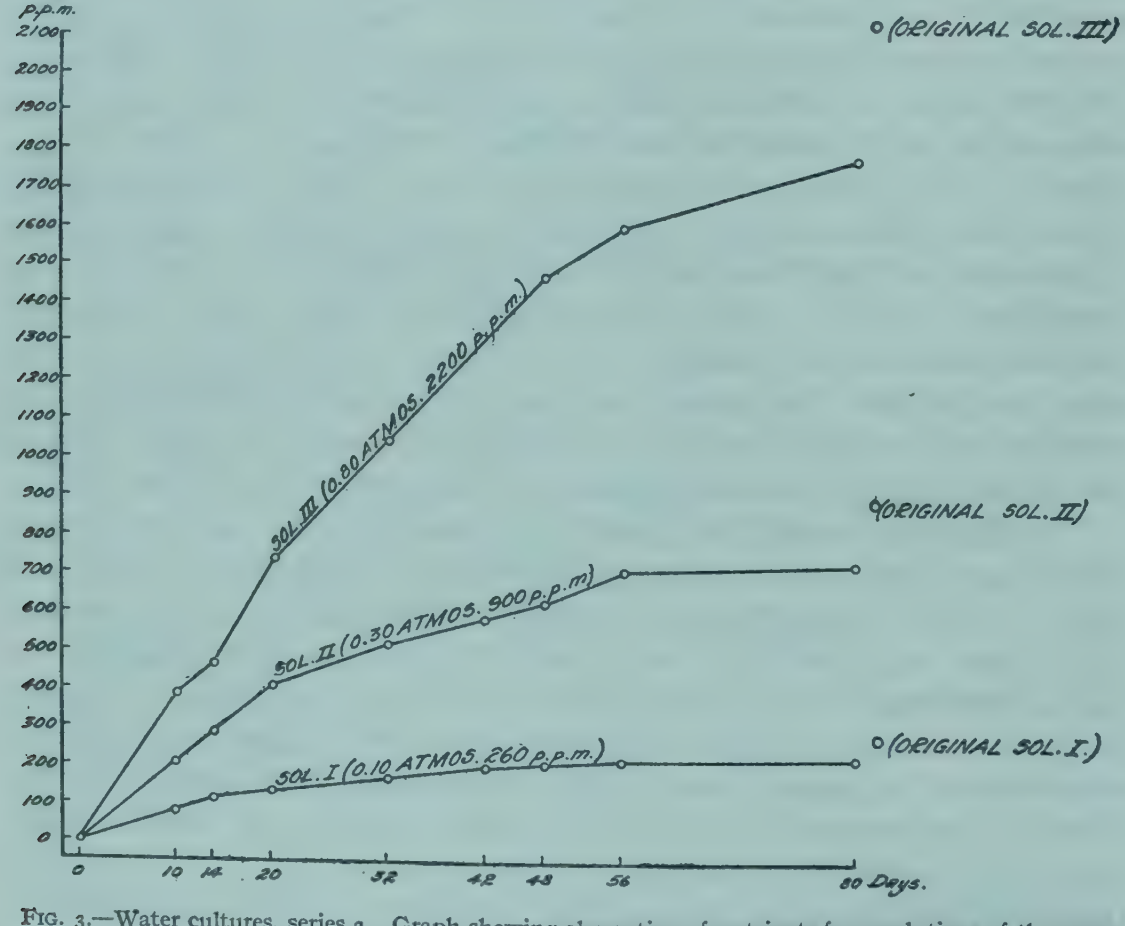

FIG. 3.—Water cultures, series 2. Graph showing absorption of nutrients from solutions of three concentrations during latter part of growth cycle. Solutions not changed during the final 80 days.

The absorption was followed each week by means of conductivity measurements, by removing portions of solutions from the culture bottles after adding water to restore the original volume. The solution was thoroughly mixed by passing a current of air through it. After the conductivity was determined the samples were returned to the original bottles. The course of absorption is graphically indicated in figure 3. The data are calculated to percentages of total electrolytes absorbed

on the basis of the original solution. At the end of the experiment composite samples were made of each solution, and these were analyzed for the important elements present. The results are shown in Table VII.

TABLE VII.—*Analyses of nutrient solutions of different concentrations after growth of plant*

WATER CULTURES, SERIES 2

Approximate concentration of solution.	Osmotic pressure.	Composition of solution after growth of plants.						Percentage of absorption by plant.					
		NO_3.	PO_4.	K.	Ca.	Mg.	SO_4.	NO_3.	PO_4.	K.	Ca.	Mg.	SO_4.
P. p. m.	*Atmos.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*						
260	0.10	0	3.1	4.3	11.5	2.7	7.4	100	76	87	66	78	85
900	.30	0	5.4	12.2	35.0	12.0	38.3	100	88	89	70	69	75
2,200	.80	12.5	1.8	43.7	87.0	35.0	105.0	99	98	85	70	64	73
[a] 1,300	.45	0	1.4	6.0	63.0	13.0	42.0	100	99	96	62	60	68

[a] Solutions not changed for 16 weeks.

Absorption continued until a large percentage of the total ions present were absorbed. In two cases the NO_3 ion was completely removed. The Ca, Mg, and SO_4 were absorbed in lesser percentage than the NO_3, PO_4, and K. The percentages of absorption for the given ions were not dissimilar in the three concentrations; therefore, under these conditions, the total absorption was approximately proportional to the concentration of ions present in the original solution. In the supplementary cultures in which the solutions were not changed between the ages of 6 weeks and maturity, practically all of the NO_3, PO_4, and K have been removed from the solution. It is interesting to note, however, that 100 per cent removal is effected only in the NO_3 ion. This fact may perhaps be explained by the nature of the metabolic processes in the plant. The NO_3 ion undergoes complete chemical transformation, and at certain stages may disappear entirely from the sap, while the other ions are always present. Thus the equilibrium conditions would differ in the two cases.

In the third series of water cultures the procedure was varied by changing the nutrient solution regularly each week throughout the whole growth of the plant, for 15 weeks. At each change of solution conductivity measurements were made, and the average absorption of electrolytes for the week was computed. The plants were grown two in each jar of approximately 1,000-cc. capacity. After 10 weeks part of the jars were changed to a concentration of 0.10 atmospheres, while the other were continued with concentrations of 0.90 atmospheres. In each case both neutral (P_H 6.5 to 6.8) and acid (P_H 5.1 to 5.5) solutions were compared under otherwise similar conditions. The composition of the solutions is given in Table VIII.

TABLE VIII.—*Composition of nutrient solutions*

WATER CULTURES, SERIES 3

Osmotic pressure.	P_H.	NO_3.	PO_4.	K.	Ca.	Mg.	SO_4.
Atmos.		*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
0. 10	5. 5	88	14. 4	19. 8	23. 7	8. 9	27. 5
. 10	6. 5	80	10. 6	20. 3	22. 9	9. 4	31. 6
. 90	5. 1	1, 100	180. 0	248. 0	296. 0	90. 0	344. 0
. 90	6. 8	1, 000	132. 0	252. 0	286. 0	102. 0	395. 0

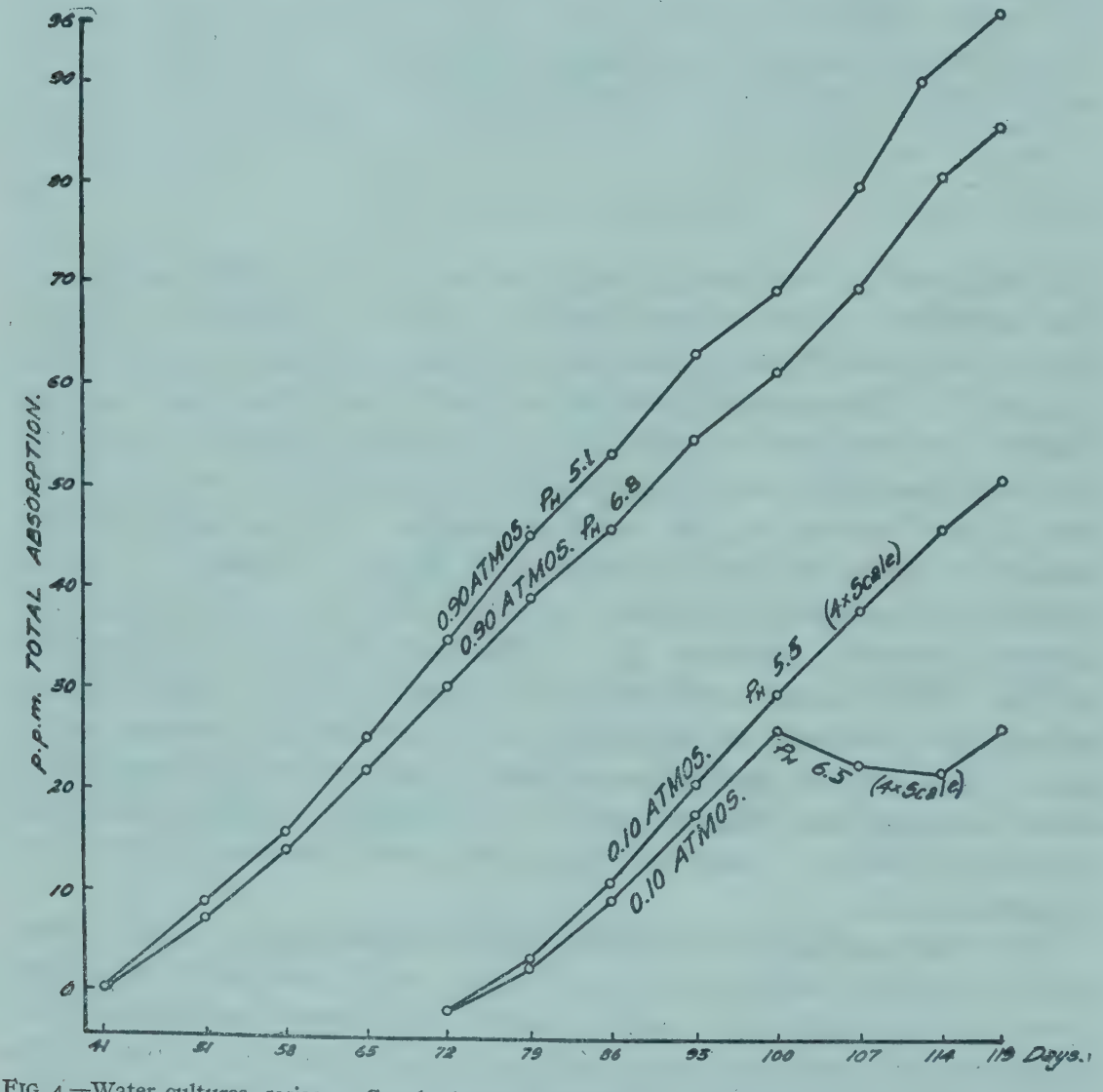

FIG. 4.—Water cultures, series 3. Graph showing absorption of nutrients from solutions of two concentrations and two reactions. Solutions changed weekly.

In figure 4 the course of absorption is shown graphically, and comparisons may be made with figure 3. The absorption is plotted for convenience as parts per million of total electrolytes absorbed, the calculations being based on the conductivities of the solutions. These graphs demonstrate clearly that in solutions of 0.10 atmospheres concentration (P_H 5.5), of 0.90 atmospheres (P_H 6.8), and of 0.90 atmospheres (P_H 5.1) absorption continues in a fairly uniform way for the entire time. The

solution of 0.10 atmospheres concentration (P_H 6.5), however, gives a different type of curve. During the fourteenth to sixteenth weeks, electrolytes instead of being absorbed were returned to the nutrient solution. During the final week absorption was again resumed.

These measurements are particularly interesting, since Burd (7) has observed an analogous phenomenon in the absorption of barley plants grown in soils. In this investigation plants were cut and analyzed at various stages of growth in such a way that the total content of the important elements could be calculated on the basis of an average plant. At a certain period in the growth cycle, which coincided approximately with the period of lowest concentration in the soil solution, there was a marked loss of K and N from the tops of the plants together with a small loss of Ca, Mg, and PO_4. After several weeks, absorption, or at least a return of elements to the tops of the plants, again took place. Although in a soil experiment it is not possible to recover roots quantitatively, it is thought probable that a considerable portion of the elements lost from the tops returned to the soil. Certainly in the solution-culture experiment, electrolyte concentration increased in the solution mentioned. This did not occur, however, in those solutions in which the higher concentration was maintained nor in the solution of low concentration with an acid reaction. The latter fact may receive a possible explanation in some experiments dealing with the effect of hydrogen-ion concentration, to be discussed later in the article.

The plants grown in this series, as in series 2, remained green in the higher concentration. The heads ripened, but a good deal of the grain had a shrunken appearance. The yields in terms of air-dry weight are as follows:

Osmotic pressure.	P_H.	Average weight per plant.[a]	
		Straw.	Heads.
Atmos.		*Grams.*	*Grams.*
0.10	5.5	13.0	7.7
.10	6.5	12.8	7.4
.90	5.1	19.3	8.2
.90	6.8	17.5	7.0

[a] Based on 10 plants.

The dry weight of the straw is somewhat greater where a higher concentration of solution has been maintained, but the yield of heads is not significantly different.

WATER CULTURES, SERIES 4

This series had for its object the comparison of yields in solutions of various concentrations and the determination of the absorption of each ion from the different solutions during a given period of time. One-liter bottles were employed with 2 plants in each bottle. Solutions were

changed every two days in the lower concentrations and every three days in the others. Ten bottles with 20 plants were used for each solution. Four concentrations were tested 0.07, 0.58, 0.90, and 1.70 atmospheres. In each concentration, solutions of both acid and neutral reaction were subjected to experiment. The composition of the solutions was such that in each concentration the acid and neutral solutions had practically identical freezing-point depressions and as nearly as possible the same ionic ratios. The exact composition of the solutions is given in Table VIII. The plants were grown out of doors in a uniform light from June 26 to August 22. The bottles were so arranged and changed in position that the light and temperature conditions were essentially the same for all cultures.

When the plants had grown for 6 weeks, the absorption study was made for a period of 72 hours. The original solutions were analyzed for Ca, Mg, PO_4, NO_3, K, and SO_4; and the same solutions after contact with the plant and after they had been made up to original volume were again analyzed. The differences between the two analyses represent the change in concentration due to 72 hours' absorption by the plant, expressed as parts per million of the various ions. The total quantities removed are obtained by multiplying the parts per million change by the volume of the solution.

The data incorporated in the above table furnish a basis for a number of interesting suggestions concerning absorption. In the first place it is noted that in the lowest concentration the percentage of absorption for all elements is much greater than in the two highest concentrations. This is in accord with the results from the first series of water cultures. The total quantities absorbed per plant are much greater in the concentration of 0.90 atmosphere as compared with 0.07 atmosphere, but in the highest concentration there is no corresponding increase and in a number of instances there is a decrease. If a large number of solutions were employed with small increments in concentration, we may infer that the total quantities absorbed would increase up to a certain total concentration and then would remain constant or decrease. Since the percentage of absorption, however, might be different there would not be necessarily a direct proportionality between the concentration of each ion and the quantity absorbed. Some data are cited by Pouget and Chouchak (*34*) in support of the assertion that NO_3 absorbed by wheat seedlings is proportional to the concentration up to a certain point. This could not be finally decided except under conditions which permitted absorption studies with controlled conditions of concentration over various time periods.

TABLE IX.—*Absorption from solutions of various concentrations and reactions in a 72-hour period by plants 7 weeks old*

WATER CULTURES, SERIES 4

COMPOSITION (IN PARTS PER MILLION OF ORIGINAL SOLUTIONS)

Osmotic pressure.	P_H.	NO_3.	PO_4.	K.	Ca.	Mg.	SO_4.
Atmos.							
0.07	5.5	88	14.4	19.8	23.7	8.9	27.5
.07	6.5	80	10.6	20.3	22.9	9.4	31.6
.88	5.1	1,100	180.0	248.0	296.0	90.0	344.0
.89	6.8	1,000	132.0	252.0	286.0	102.0	395.0
1.72	4.9	2,200	354.0	500.0	560.0	183.0	697.0
1.70	6.1	2,000	222.0	501.0	536.0	202.0	770.0

ABSORPTION (IN PARTS PER MILLION OF SOLUTION)

0.07	5.5	88	12.9	19.0	10.2	4.2	9.8
.07	6.5	80	9.4	19.4	7.4	3.4	11.7
.88	5.1	421	71.0	74.0	52.0	8.0	34.0
.89	6.8	244	35.0	77.0	22.0	22.0	26.0
1.72	4.9	322	124.0	38.0	48.0	17.0	39.0
1.70	6.1	345	78.0	61.0	48.0	21.0	45.0

NUMBER OF GRAMS ABSORBED PER PLANT)

		Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
0.07	5.5	0.044	0.006	0.010	0.005	0.002	0.005
.07	6.5	.040	.005	.010	.004	.002	.006
.88	5.1	.210	.035	.037	.026	.004	.017
.89	6.8	.122	.017	.038	.011	.011	.013
1.72	4.9	.161	.062	.019	.024	.008	.019
1.70	6.1	.172	.039	.030	.024	.010	.022

PERCENTAGE OF ABSORPTION

0.07	5.5	100.0	89.6	96.0	43.0	47.2	35.7
.07	6.5	100.0	88.6	95.6	32.3	36.2	37.0
.88	5.1	38.2	39.5	29.8	17.6	8.9	9.9
.89	6.8	24.4	26.5	30.6	7.7	21.6	6.6
1.72	4.9	14.7	35.0	7.6	8.5	9.3	5.6
1.70	6.1	17.2	35.1	12.2	9.0	10.4	5.9

In the solutions of 0.07 atmospheres concentration, it is significant that all the NO_3 ions and nearly all the K and PO_4 ions were absorbed within 72 hours. It is not possible to say from these data just how the absorption was distributed over the period of time in question, but it is logical to suppose that the total quantities absorbed per hour decreased until finally in the NO_3 ion every trace had disappeared from the solution.

The air-dry yields are given in Table X. Plants from each jar were weighed separately in order to gain some idea of the mean deviation and thus provide a basis for judging the significance of the results.

TABLE X.—*Yields of tops and roots*

WATER CULTURES, SERIES 4

[Plants grown for eight weeks]

Osmotic pressure.	P_H.	Average air-dry weight of tops per plant.	Mean deviation for 18 plants.	Average air-dry weight of roots per plant.	Mean deviation for 14 plants.
Atmos.		*Grams.*	*Grams.*	*Grams.*	*Grams.*
0.07	5.5	2.9	±0.15	0.56	±0.05
.07	6.5	2.9	± .15	.62	± .03
.58	5.1	7.4	± .50	1.19	± .21
.56	6.8	7.0	± .60	1.24	± .15
.88	5.1	8.1	±1.00	1.39	± .19
.89	6.8	5.8	±1.00	.95	± .13
1.72	4.9	3.8	± .60	1.09	± .15
1.70	6.1	4.5	± .50	.92	± .17

The yields from concentrations of 0.07 atmospheres and 1.70 atmospheres are decidedly inferior to those from concentrations of 0.58 atmospheres and 0.90 atmospheres. Between the two latter there is no significant difference. The yields of plants from the acid solution are at least equal and possibly superior to those from the neutral solution. Certainly there is no evidence of inhibition in the acid solutions, except with the highest concentration. This is a super-optimum concentration for both acid and neutral solutions; but in the acid solution of this highest concentration the roots were distinctly injured, while those in the corresponding neutral solution had a more normal appearance. In the other solutions of similar hydrogen-ion concentration the roots were quite uninjured. In other words, the injury referred to was the resultant effect of the hydrogen-ion concentration and super-optimum total concentration, or more specifically, perhaps, due to the high concentration of PO_4 in the acid solution. A much larger quantity of PO_4 was absorbed in the latter case.

It should be emphasized in connection with the relative yields that the most dilute solution was exhausted of its NO_3 in less than 72 hours. The question then arises, whether the diminution in yield might not be due to a deficiency in total quantity of one or more ions rather than to sub-optimum concentration.

This suggestion led to a further water-culture experiment, series 5. Twenty plants were grown in bottles of 2,200–cc. capacity with only 1 plant in each bottle. Solutions were changed, after the first few weeks, about five times each week. In this way, as analysis showed, the solutions were maintained practically constant. The composition of the solutions was similar to that described before, two concentrations being used of 0.07 atmospheres and 0.58 atmospheres. After seven weeks the plants were cut and weighed in the air-dry state. The concentration of 0.07 atmospheres gave a yield of 0.60±0.08 gm. of tops and 0.20 gm. of

roots per plant, and the 0.58 atmospheres concentration a yield of 0.66 ± 0.08 gm. of tops and 0.24 gm. of roots. Between these figures there is no significant difference, although in water cultures, series 1 and series 4, similar solutions gave distinctly different yields. To verify these results finally it will be necessary to repeat the experiment at a more favorable season of the year; but we may reasonably conclude, taking all the experiments into consideration, that with a solution of the general composition described above the optimum concentration for the barley plant—defined as the least concentration producing a yield equal to any higher concentration—is not higher than 0.60 to 0.90 atmospheres and may be less than 0.10 atmospheres. This point is to be considered more critically in the final discussion.

In one of the preceding experiments reference was made to the observation that under certain conditions electrolytes might leave the plant and return to the solution. In order to gain some additional insight into this process, a number of plants which had grown for 6 weeks in favorable nutrient solutions were transferred to various very dilute nutrient solutions after washing the roots thoroughly in distilled water. The results are presented in Table XI, in which comparisons are made between the resistances of original solutions and the same solutions after contact with the plant.

TABLE XI.—*Absorption by plants from dilute solutions*

Concentration of nutrient solution.	Resistance of original solutions.	Resistance of solutions after contact with plants for—							
		4 days.	7 days.	14 days.[a]	19 days.	28 days.	35 days.	42 days.	49 days.
P. p. m.	*Ohms.*	*Ohms.*	*Ohms.*	*Ohms.*	*Ohms.*	*Ohms.*	*Ohms.*	*Ohms.*	*Ohms.*
25	3,380	1,190	1,290	5,940	16,300	25,200	5,300	5,630	5,390
75	1,150	850	1,090	1,620	5,580	15,200	16,500	3,420	9,230
150	630	530	600	1,050	1,630	6,540	8,290	10,050	10,400
300	340	400	475	610	860	3,180	5,800	4,590	6,130
500	210	275	390	350	470	1,880	4,600	7,170	6,130

[a] Solutions changed.

In the two most dilute solutions the electrolyte concentration was increased, and from the three higher concentrations absorption took place. Later absorption occurred from all solutions, so that in several cases the resistance of the solutions became about the same as that of the distilled water used. Subsequently there was a further excretion of electrolytes from the plant. True and Bartlett (49, 50, 51) have described phenomena similar to these, working with partial nutrient solutions in very great dilution.

True (*48*) has also pointed out that distilled water may be injurious because of the elements leached out from the plant. In order to ascertain which elements would leave the plant we have transferred a number of

plants as described above to distilled water and after several days have analyzed the solution. The composition was found to be as follows:

	P. p. m.		P. p. m.
NO_3	None.	Ca	19.0
PO_4	5.7	SO_4	None.
K	1.2	Mg	1.7

Ca and PO_4 were thus the principal elements leached out. It will be recalled that in sand cultures of series 2 and 3 considerable accumulation of Ca and PO_4 occurred in the roots.

In order to compare absorption at different periods of the growth cycle, analyses of solutions were made after the plants had absorbed for a period of a week from solutions of 0.90 atmospheres concentration with reactions of P_H 5.1 and 6.8. The periods chosen were 4 to 5 weeks, 6 to 7 weeks, and 11 to 12 weeks. In Table XII are shown the total quantities per plant and percentages of the various elements absorbed.

TABLE XII.—*Absorption of elements at different stages of growth*[a]

[Absorption period seven days]

Period of growth.	Osmotic pressure.	P_H.	NO_3.	PO_4.	K.	Ca.	Mg.	SO_4.
	PARTS PER MILLION OF SOLUTION ABSORBED							
	Atmos.							
4 to 5 weeks	0.88	5.1	384	60	90	48	8	42
Do	.89	6.8	157	43	65	28	9	23
6 to 7 weeks[b]	.88	5.1	536	108	126	92	20	71
Do	.89	6.8	416	61	125	61	16	65
11 to 12 weeks	.88	5.1	545	146	107	84	17	72
Do	.89	6.8	325	93	112	74	23	81
	NUMBER OF GRAMS ABSORBED PER PLANT							
4 to 5 weeks	0.88	5.1	.192	0.030	0.045	0.024	0.004	0.021
Do	.89	6.8	.078	.021	.032	.014	.004	.011
6 to 7 weeks	.88	5.1	.268	.054	.063	.046	.010	.035
Do	.89	6.8	.208	.030	.062	.030	.008	.032
11 to 12 weeks	.88	5.1	.272	.073	.053	.042	.008	.036
Do	.89	6.8	.162	.046	.066	.037	.011	.040
	PERCENTAGE OF TOTAL QUANTITY ABSORBED							
4 to 5 weeks	0.88	5.1	34.8	34.6	34.7	15.9	9.0	12.2
Do	.89	6.8	15.7	37.7	23.0	9.7	9.3	6.0
6 to 7 weeks	.88	5.1	48.6	61.8	48.0	30.5	22.5	20.6
Do	.89	6.8	41.6	53.5	44.2	21.2	16.5	16.9
11 to 12 weeks	.88	5.1	49.4	81.0	43.1	28.4	18.8	20.9
Do	.89	6.8	32.5	70.5	44.5	25.9	22.6	20.5

[a] Studies made on composite solutions, each representing 20 plants with 2 plants per liter of solution.
[b] Computed from 9-day absorption period.

It will be noted that the total quantities and percentages were considerably greater in the two later periods as compared with the first period. Absorption in the eleventh to twelfth weeks was not greatly different from that in the sixth to seventh weeks, except that in the latter period the absorption of PO_4 was decidedly increased. In each period the absolute quantity of PO_4 absorbed from the acid solution exceeded that from the

neutral solution. This is also true of several other elements, particularly NO_3, although the concentration of NO_3 was approximately the same in the original acid and neutral solutions. The concentration of PO_4 was, of course, greater in the original acid solution.

THE EFFECT OF REACTION ON GROWTH AND ABSORPTION

As was stated earlier in this article, all the nutrient solutions were controlled with respect to hydrogen-ion concentration. This subject has received some previous study in this laboratory. In an earlier investigation (*16*), it was shown that barley seedlings were not injured nor inhibited by a hydrogen-ion concentration of P_H 5. More recent work has confirmed these views for barley plants at all stages. Furthermore, certain reports from the field regarding peat soils have indicated that acidity of an intensity represented by P_H 4.8 or 5 is not injurious to a large number of common agricultural plants.

In some of these investigations the interesting fact was discovered that in none of the nutrient solutions examined was there any tendency for the plant to produce an excessive concentration of hydrogen or hydroxyl ion, but that the opposite was true. Both alkaline and acid solutions were brought approximately to the neutral point as a result of absorption by the plant. In the water cultures of series 3, hydrogen-ion concentrations were determined by colorimetric methods at each weekly change of the nutrient solution, as follows:

TABLE XIII.—*Hydrogen-ion concentration of nutrient solutions after absorption by plant for one week*

WATER CULTURES, SERIES 3

Osmotic pressure of original solution.	P_H value of original solution.	P_H value after absorption for—								
		58 days.	65 days.	72 days.	79 days.	86 days.	93 days.	100 days.	107 days.	114 days.
Atmos.										
0.10	5.5	.....	.....	.....	7.2	.....	6.3	6.5	6.5	6.8
.10	6.5	.....	.....	.....	7.2	.....	6.5	6.5	6.5	6.8
.90	5.1	6.8	7.0	7.2	7.2	7.2	7.2	6.8	7.0	6.8
.90	6.8	6.8	7.0	7.2	7.2	7.4	7.0	6.8	7.0	6.8

At the end of the period of contact with the plant all solutions gave an almost neutral reaction. Control experiments showed that no appreciable change of reaction was due to the glass in contact with the solutions. By referring to Tables IX and XII one may compare the changes of hydrogen-ion concentration with the removal of the various ions from solution. In general it may be said that absorption is so altered in the acid solutions that larger percentages of a number of ions are absorbed, as compared with the corresponding neutral solutions. NO_3 and Ca are particularly affected. In the solution of 0.90 atmospheres concentration and P_H 5.4 a considerably higher percentage of PO_4

was absorbed. In all cases the total quantity of PO_4 absorbed was greater with the acid solutions. The increased absorption from acid solutions is also definitely indicated in figure 4. Here the total ionic absorption as determined by conductivity measurements was consistently greater in the solutions of P_H 5.1 as compared with solutions of P_H 6.8, both of practically the same concentration.

Even when solutions of single salts are used the absorption by the plant does not bring about an unfavorable condition of acidity or alkalinity. To determine the nature of such absorption, plants were grown in favorable nutrient solutions until they had reached a stage of active absorption. They were then transferred to solutions of sodium nitrate ($NaNO_3$), potassium chlorid (KCl), potassium sulphate (K_2SO_4), magnesium sulphate ($MgSO_4$), potassium phosphate (K_3PO_4), and ammonium chlorid (NH_4Cl). From the $NaNO_3$ solution a greater percentage of NO_3 than Na was absorbed, but equilibrium was restored by the formation of carbonic acid (HCO_3) ion. This in equilibrium with carbon dioxid (CO_2) gave to the solution an approximately neutral reaction, nor did other solutions tested acquire an excessive concentration of hydrogen or hydroxyl-ion. K and Cl were found to be absorbed in equivalent quantities. The high alkalinity of the potassium phosphate (K_3PO_4) solution was reduced to a condition of slight alkalinity. Further details of these experiments are reported elsewhere (*18*).

In the complete nutrient solution it is impossible to say exactly what ions and undissociated salts are present before and after absorption by the plant. Such a system, with its various hydrolyzable salts, is very complex. Calculations of reacting values for the various elements present indicate that an excess of acid radicles have been absorbed by the plant, in greater degree from the solutions of acid reaction. These have attained a practically neutral reaction after contact with the plant, as noted above. Since the solution must remain balanced with respect to positive and negative ions, some other ions must have been formed in the solution. Where NO_3 is absorbed in large percentage it is probable that CO_3 or HCO_3 become important constitutents of the solutions. A small quantity of silicate radicles is also found, derived from action on the glass. The resultant reaction is due to the particular state of equilibrium existing among all these constituents; and while we may determine the hydrogen-ion concentration with considerable accuracy, the data at present available do not enable us to determine the exact relations between the different components of the system.

THE EFFECT OF THE NUTRIENT SOLUTION ON THE CELL SAP

That the concentration of the nutrient medium is reflected to a certain extent in the cell sap has been shown by McCool and Millar (*30*). In another article by the present author [1] are described determinations

[1] In course of publication in Bot. Gaz.

on the sap expressed from the tops and roots of the plants grown in water cultures, series 4. Measurements were made of hydrogen-ion concentration, depression of the freezing point, and specific conductivity. It was shown that the total osmotic pressure and conductivity increased with increasing concentration of the nutrient solution. Analyses of the sap of barley plants gave evidence of a very high concentration of ions, many times greater than that found in the soil or nutrient solution. The following figures give an idea of the magnitudes concerned:

	PO^3.	K.	Mg.	Ca.	NO^4.	Total N.
Parts per million of sap ...	800 to 2,000	5,400 to 6,100	270 to 340	600 to 1,000	700 to 2,500	600 to 1,400

The hydrogen-ion concentration of the sap pressed from the tops of barley plants displayed a great constancy. The P_H value of 6.1 was practically the same in the sap of plants from sand, water, and soil cultures, even when the osmotic pressures and conductivities differed greatly.

GENERAL DISCUSSION

Having now presented the data obtained in the course of these investigations, it becomes necessary to survey the experiments as a whole, with the intention of considering certain principles of plant nutrition. It will also be desirable to correlate the present results with those of other investigators. No attempt will be made, however, to give any complete bibliography of the general subject. This has already been done by Tottingham (*45*), Shive (*39*), and Pantanelli (*32*), among others. Only such citations will be made as bear directly upon the questions considered in this article.

Before proceeding further in this discussion, we wish to call attention to one very serious deficiency in the fundamental data necessary to any extension of plant nutrition studies. There appear to be no adequate and systematic experiments capable of showing the variability of plants under favorable and unfavorable conditions, yet such a basis of calculation is indispensable to any proper interpretation of yields, when small variations of nutrient solutions are concerned. This factor has been called to the attention of the author by the work of Waynick (*53*), who has shown how very erroneous may be the conclusions derived from soil studies when the statistical method of interpretation is neglected. These criticisms apply also, at least in large measure, to many investigations in plant nutrition. In the interpretation of the data obtained in our investigations there has been no intention of assigning relative values to a large number of solutions. The whole purpose has been to determine the general magnitudes involved and to explain something of the nature of the course of absorption by the plant, with the hope of gaining further knowledge of the principles involved. It

would seem highly desirable that some sort of critical basis be established before attempting more highly specialized experiments dealing with small variations.

It is of course quite obvious that no control of the soil is possible sufficient to elucidate the fundamental points in plant nutrition. Recourse to water and sand cultures is essential, without question. We may, however, regard the soil as the natural habitat of agricultural plants, and when a high yield is obtained we are justified in assuming that the particular conditions that obtain in the soil solution are not unfavorable to the development of the plant. Any definite information, therefore, which may be obtained regarding the soil solution will serve as a guide to at least one set of favorable factors. Very little is known of the soil solution, but the method perfected by Bouyoucos and McCool (*3*) has enabled us to ascertain with fair accuracy the total osmotic values. This method has been applied in soil investigations conducted by this laboratory, determinations being made at frequent intervals throughout the season. The results were quite definite in showing that about 10 weeks after planting the barley crop had diminished very significantly the concentration of the soil solution. Water extracts indicated that by this particular period very little NO_3 remained in the soil solution. Moreoever, high yields of barley and wheat have been obtained on soils whose solutions at no time during the growth cycle had a concentration greater than 0.5 atmospheres. Similar relations have been observed for a number of other plants. We may conclude from these soil investigations that the plant does not necessarily require a concentration of the nutrient medium higher than the one stated above, and that it is not necessary that the concentration or large supply of NO_3 be maintained during the latter part of the growth cycle.

These facts were made the basis of several water- and sand-culture experiments already described. It became evident from these that at least for the barley plant the normal cycle of development did not require that the concentration of the nutrient solution be maintained after nine weeks for the climatic conditions under which the experiment was conducted. A longer continued maintenance of the concentration leads to no important increase in yield but does cause the plant to attain a very much higher percentage of certain elements. Also when the supply of NO_3 and other elements is constantly replenished, the plant may remain green almost indefinitely. In the soil the particular cycle of development of the plant is related to the diminution in the concentration or total supply of elements in the soil solution, and this diminution itself has been brought about to a large extent as a result of absorption by the plant. We may infer, then, that the most important condition for a high yield, in so far as the soil solution is concerned, is an adequate concentration and supply of nutrients during the first half

of the growth cycle. If the concentration or supply is either sub-optimum or super-optimum during this period, no subsequent favorable condition is likely to produce maximum yield. An inhibitive concentration for barley either in water, sand, or soil cultures is not extremely high, possibly less than 2.5 atmospheres. The minimum concentration giving a maximum yield is low, though the magnitude can not be exactly stated at present. It may not be more than that represented by 0.1 atmospheres osmotic pressure.[1]

Some recent work of Davidson and LeClerc (*9*) bears on certain of the above statements. These investigators give evidence that the greatest increase in yield of wheat is obtained when soil fertilization is accomplished during the first stage of growth, a slighter effect is produced during the second stage, and no effect during the third. An increased percentage of N in the grain occurs when fertilization takes place during the second stage. During the third stage no effect is produced. It is possible that the N content of the grain may be influenced by the length of time during which nitrification continues in the soil, and this latter process may in part be governed by climatic conditions. Very marked seasonal fluctuations in NO_3 have been noted by Stewart (*42*) in soils kept always at optimum moisture content and in an excellent state of cultivation.

Finally, in the consideration of the relation of the soil solution to plant growth it should be pointed out that the interpretation of soil experiments in terms of concentration of the soil solution requires recognition of the fact that the growth of the plant is affected by the properties of the solution in actual contact with the absorbing root membranes. The degree to which nutrient elements are maintained at a favorable concentration in this effective solution must depend upon the rate of diffusion and upon the potentiality possessed by the soil for constant renewal of the solution as elements are absorbed by the plant. The question of diffusion has been discussed by Russell (*36*), while Burd (*6*) has shown the necessity of taking into account the renewing power of the soil. It follows that conclusions drawn from determinations on samples of the whole mass of soil do not imply an exact knowledge of conditions in the solution from which the plants are actually absorbing.

The question of the optimum concentration of solution for barley, wheat, and other grains has been discussed in a number of articles with considerable disagreement in the conclusions. It may be well to define the conditions necessary for the determination of the effect of concentration on plant growth. In such a study it is essential that the concentration of the solution be maintained constant at all times. In other words, as absorption takes place the solution must be renewed. Ideally this can be accomplished only by a continuous flow. Such a technic is

[1] Later experiments show that this concentration is somewhat less than optimum when light and temperature conditions are highly favorable.

usually impracticable, so it is necessary to approximate the requisite condition by changing the solution at more or less frequent intervals. In order to determine exactly what has been the average condition of the solution between changes, analyses must be made as in the previously described experiments. Obviously the extent of change in the solution will depend upon the rate of absorption by the plant and also upon the concentration and total volume of solution per plant. Consequently, actual determinations of the quantity of each ion absorbed must be the basis for the selection of suitable culture vessels, number of plants, and times of changing solution. If too small a total supply per plant of any element is present, all or nearly all the ion in question may be removed from the solution in the interval between changes of solution. Since all of the ions are not absorbed in equal percentages, not only the total concentration of the solution but the ratio between ions will be changed. The average composition of the solution will depend then upon the particular set of empirical conditions chosen. How very important these considerations are is indicated by all the absorption studies of this investigation. It will be recalled, for example, that when two barley plants seven weeks old were placed in 1 liter of a solution of about 200 parts per million total concentration, in less than 72 hours every trace of NO_3 and over 90 per cent of the K and PO_4 were removed from the solution. It is quite clear from such an experiment that absolute quantities rather than concentration may have been the limiting factor. In fact a later experiment indicated that such was the case.

Brenchley (*5*) grew barley and wheat plants for seven weeks in solutions of 3,000, 600, 300, and 150 parts per million. The solutions were changed every four days. She concluded that the lower concentrations are sub-optimum and criticised Stiles' (*43*) results. The latter grew single plants in 1,200-cc. bottles for six weeks, changing the solution every three or five days and using concentrations of 1,750 parts per million and one-fifth, one-tenth, and one-twentieth of that concentration. He did not find a significant difference in yield, although there was some falling off in the lowest concentration.

Tottingham (*45*), as a result of various experiments by the water-culture method, using 250- or 400-cc. bottles with six plants to a bottle for a growth period of 23 days, decided on a concentration of 2.5 atmospheres as optimum, though he recognized that a less concentrated solution might give an equal yield.

Shive (*39*), with a technic similar to that employed by Tottingham except for the use of a 3-salt nutrient solution, came to the conclusion that 0.1 atmosphere was sub-optimum concentration and assumed the optimum concentration to be 1.75 atmospheres. This concentration has since been adopted by other investigators as optimum. In Shive's (*40*, *41*) sand-culture experiments about 250 cc. of nutrient solution

remained in each jar after changing solutions. There were three to five plants in each jar.

Lyon and Bizzell (*25*) found that wheat seedlings gave increased growth with increasing concentrations from 83 to 4,525 parts per million total salts. They used 120-cc. bottles in the water-culture series.

Bouyoucos (*2*) also states that increased yields occur with increasing concentrations up to 4,500 parts per million. In his experiments 120-cc. bottles were used with four plants in each bottle. The period of growth was three or four weeks. Solutions were changed once each week.

Other experiments similar to these have been carried out, but these citations will suffice for the present purpose. The point which it is desired to emphasize now has been clearly stated previously by Stiles (*44*) in his criticisms of Brenchley's conclusions, but these criticisms have apparently been neglected in later work. In all of the above-mentioned experiments no sufficient distinction has been made between supply of nutrients and concentration of nutrients. If we compare the quantities of nutrients per plant available between changes of solutions with quantities of nutrients actually absorbed, as shown by data given in this article, we must conclude that in many cases the total supply may have fallen far short of the requirements, so that the solutions were constantly undergoing a great change, due to absorption. Moreover the relative changes may be very different in different solutions. For example, in those solutions in which only one-tenth of the total concentration was due to $Ca(NO_3)_2$ the NO_3 supply conceivably may have been entirely insufficient, whereas in solutions with a higher ratio of $Ca(NO_3)_2$ the supply of NO_3 may not have been exhausted. If all the ions were not absorbed in the same proportion, the result would be a continuously varying solution, with regard to both total concentration and ionic ratios. Without knowing precisely the nature of these changes it would seem difficult to interpret the results in terms of ionic ratios. Perhaps it is significant that in many cases the areas of low yields in the triangular diagrams have been found near the line of least $Ca(NO_3)_2$. NO_3 is the element of greatest importance quantitatively. In the following table some estimates are made of the total volume of solution per plant necessary to furnish total quantities of nutrients equal to those absorbed by the plant, under conditions permitting good yields of crop.

As another basis of calculation we may use the data for the sand cultures of series 1. It can hardly be denied in this case that a lack of total NO_3, K, PO_4 were limiting factors, and that the total yield per plant was greatly reduced by reason of these deficiencies. Yet even under these conditions the total quantities of nutrients found in each plant were 0.15 gm. K, 0.15 gm. PO_4, and 0.30 gm. N calculated to NO_3. To supply these quantities of NO_3 would require 1 liter per plant of a nutrient solution containing 300 parts per million of NO_3 or 20 changes

on basis of 50 cc. per plant. If in addition it were desired to maintain the solution at approximately the same concentration and composition at all times, many times this number of changes of solution would be necessary. In this general connection it may be noted that Trelease and Free (47) have given brief mention to an experiment in which it was found that higher yields were obtained in cultures with a continuous flow of solution.

TABLE XIV—*Approximate volumes of solution equivalent to total quantity of NO_3 absorbed per plant*

[Nutrient solution containing 300 parts per million NO_3]

Age of plant.	Solution required per plant in three days, based upon absorption by plant from soil.	Solution required per plant in three days, based upon absorption by plant in water cultures from nutrient solution of 2,200 parts per million concentration.
Weeks.	*Cc.*	*Cc.*
4 to 5	100	260
6 to 7	300	380
11 to 12	100	380

On basis of 50 cc. per plant, solutions would have to be changed from once daily to three or four times daily to provide the quantity of N calculated from above absorption studies. If K or PO_4 were present in low concentration, large volumes of solution would also be necessary to supply these elements in the quantities capable of absorption. To maintain approximate constancy in solutions, very much larger volumes than the foregoing might be required.

It is of course unjustifiable to apply at all rigidly absorption data obtained in one set of plant studies to another set, for the reason that temperature or light conditions may in some cases be so unfavorable that any large growth is impossible, and as a result absorption also will be greatly diminished because the plant is stunted. Moreover, in the first few weeks of growth absorption is comparatively slight; only when plants are grown six weeks or longer will the full extent of absorption become apparent. Some experiments dealing with concentrations and ionic ratios have not been carried on for a sufficiently long time to give an adequate idea of the effects of the various solutions tested.

The statement is often made in texts on plant physiology that entirely normal plants may be grown in solution cultures, but no data are given concerning the yield per plant of grain and total dry matter. In fact, only recently have detailed results been presented of systematic experiments in which plants have been grown to maturity. In most of the experiments the data and descriptions would indicate that the plants obtained were decidedly inhibited by some factor, the total dry weight per plant, height, number of tillers, etc. being usually less than for similar plants grown under favorable conditions in the field for an equal period. Various causes might be assigned to account for the diminished yields. Obviously light or temprature may be unfavorable, and enor-

mous fluctuations may be due to these factors. Possibly other conditions related to the physical nature of the medium may have an effect. There is also a strong presumption in certain experiments that the total supply of nutrients may limit the yield in the manner just outlined. In any case it would seem necessary to determine what are the limiting factors that prevent the production of an optimum plant. It is possible to obtain in sand and water cultures plants very similar in size to those given in productive fields under equal climatic conditions. The following data are evidence of this statement.

AIR-DRY YIELD PER PLANT.

Kind of culture.	Heads.	Stems and leaves.
	Grams.	*Grams.*
Water solution	8 to 12	10 to 19
Sand	10 to 15	12 to 20
Soil	5 to 14	11 to 26

The plants of the water- and sand-culture experiments referred to above were grown out of doors under light conditions similar but inferior to those obtaining in the soil experiments. In some instances the grain presented a more shrunken appearance in the sand and water cultures, though the total yield and proportion of heads to straw are not very different.

As a corollary to the foregoing discussion the conclusion is unavoidable that no sufficient evidence has yet been adduced to show that varying salt proportions within a wide range have any significant effect on yield. The validity of such deductions can not be established until the control of the nutrient solutions is more definite and until the interpretation of the data is made with due regard to the significance of variability studies, such as those proposed by Waynick (*53*).

In connection with any critical discussion of the interpretation of data from plant nutrition studies, one other factor must be considered—the relation of solution cultures to sand cultures. Upon this point the literature is in disagreement. Bouyoucos (*2*) found that solution cultures gave a higher yield than sand cultures with the same solution. Lyon and Bizzell (*25*) observed in certain experiments that sand cultures were superior to solution cultures and advanced the hypothesis that absorption took place around the solid particles, so that the plant really obtained its nutriment from a solution of higher concentration than that added to the sand. McCall (*27*) reached an opposite conclusion. He found that a smaller yield was obtained in sand cultures and that optimum ratio of ions was changed materially. This he attributed to absorption by the sand, changing the composition and concentration of the solution available to the plant. On the other hand, Shive (*40*, *41*)

has not found that the sand had any marked influence when the same solutions are compared in sand and solution cultures. Wolkoff (*56*) confirms this point of view in general, although he finds that ammonium sulphate ($(NH_4)_2SO_4$) exhibits somewhat anomalous behavior.

During the present investigation a number of experiments were made relating to this question. Freezing-point determinations were made on nutrient solutions and on sand to which the solutions had been added to give the sand a moisture content of 15 per cent. Also solutions were allowed to stand in contact with the sand for long periods, conductivity determinations being made at intervals.

TABLE XV.—*Effect of sand on freezing-point depression and conductivity of nutrient solutions*

Freezing-point depression of solutions.	Freezing-point depression of sand immediately after adding solution.	Freezing-point depression of sand after 4 days.	Freezing-point depression of sand after 9 days.	Freezing-point depression of sand after 67 days.	Resistance of nutrient solution.	Resistance of nutrient solution with sand after 80 days.
°C.	°C.	°C.	°C.	°C.	*Ohms.*	*Ohms.*
0.025	0.032	0.027	0.028	.....	198.0	171.0
.145	.145	.149	.149	0.134	37.5	37.2

From the data given above there is no indication that the sand has appreciably altered the added solutions. To what, then, may discrepancies between sand and water cultures be ascribed? In the first place, a much more extensive root development takes place in the presence of the solid particles. This might in itself imply a different type of absorption, though plants in solution cultures are capable of sufficient absorption quantitatively under suitable conditions. Diffusion in water cultures is very rapid but must be somewhat retarded in sand culture. For this reason in the very early stages before an extensive root system has been formed solution cultures may be superior to sand cultures, while later the conditions may be reversed. Aeration is another factor which is different in the two cases. Hall and Underwood (*15*) claim that this is the chief difference between sand and water cultures. It is also conceivable that the larger root system of sand cultures might affect the growth of tops by the formation in the roots of a greater quantity of some substance accelerating growth. And finally, since even pure sand is not entirely insoluble, a greater supply of silica is available to the plant in sand culture. The exact effect of this is of course not known.

As a matter of fact, in our experiments no very striking differences are observed between sand and water cultures although it has generally been true that taller plants with a greater number of tillers are produced in sand cultures. Somewhat lower concentrations are inhibitive in solution cultures, though the magnitudes do not vary greatly, and the differences

found might reasonably be related to the extent of the root system and rapidity of diffusion. There is certainly no convincing evidence that selective adsorption by the sand plays any important rôle, although the exact relations still remain to be worked out. This can be accomplished only by comparing a sufficient number of plants under identical aerial conditions and with complete control of the solutions in all cases. Some preliminary indications suggest that the paraffin seal used in sand cultures may be slightly inhibitive to growth. This might be omitted in experiments planned to solve such questions as the above.

It will not be necessary to enlarge on the earlier discussion in this article with regard to the effect of hydrogen-ion concentration. The critical consideration of this phase of the work has already appeared elsewhere (*16–19*). It will suffice to emphasize the inapplicability of titration methods in determining the reactions of the nutrient solutions, and the fact that permeability or absorption are influenced by the hydrogen-ion concentration, although a P_H value of 5 has not been found to be inhibitive either in solution or sand culture nor in peat soils when other inhibitive factors are absent.

Finally, brief attention must be given to the methods of stating results, since these may in themselves modify the interpretation or planning of further experiments. The practice in these investigations has been to state the composition of the solutions in terms of parts per million of each ion. This seems to be the most logical scheme, since it is not possible to determine in such complex solutions the exact nature and concentration of the undissociated molecules and ions present. In most cases the dissociation values are high, and there is no satisfactory evidence to show that the ionic concentrations are not chiefly concerned in absorption by the plant. Certainly whatever the original salts used, the properties of the solution are those of the ions and molecules formed after solution has taken place. Thus we are of the opinion that it is desirable to compare solutions on the basis of parts per million of the various elements or radicles present rather than on that of molecular or osmotic fractions of the salts used in preparing the nutrient solutions. The latter method may lead to neglect of the factor of total supply of essential ions, as pointed out before.

In a recent article Tottingham (*46*) suggests as a result of some experiments performed in his laboratory that even with highly dissociated salts the particular salt used has an effect apart from the ions formed in the solution. It seems, however, that the evidence presented in support of this idea is insufficient. If a large number of plants had been used and the significance of the results evaluated, as in the previously mentioned work of Waynick (*53*), it is not apparent that the same conclusion would have been reached.

In the present investigation concentrations have been expressed for convenience in terms of atmospheres, derived from determinations of

freezing point depressions. It is realized, however, that such expressions are matters of convenience and not necessarily significant. The osmotic pressure of a solution is defined by Findlay (*10*) as the "equivalent of the hydrostatic pressure produced when the solution and solvent are separated by a perfectly semi-permeable membrane." The absorbing membranes of the roots are obviously not of this sort. They are permeable to all the ions present in varying degrees and, moreover, as shown by Osterhout (*1, p. 96–147*), the permeability is subject to change as a result of antagonistic relations among ions. It is also to be noted that the osmotic pressure and electrolyte concentration in the expressed plant sap are far greater than those of any noninjurious nutrient solution. The effects of solutions are not due to the theoretical osmotic pressures of which the solutions are capable, but rather to alterations in permeability due to specific ions or ionic relations, or to internal derangement of the metabolic processes as a result of a too great absorption of one or all ions.

SUMMARY

(1) Sand- and solution-culture experiments were carried out under conditions permitting definite control of the total concentration, composition, and reaction of the nutrient solutions. Numerous absorption studies were made throughout the growth cycle of the barley plant. Plants were obtained which were fairly comparable in size and development with those produced by a fertile soil.

(2) Marked absorption of all the nutrient elements took place at all periods up to the final stage of growth when suitable concentrations of the various ions were continuously maintained. This intense absorption during the later stages led to no important increase in yield of crop, which seemed to be conditioned in large measure on a favorable supply and concentration of nutrients during the first 8 or 10 weeks of the growth cycle.

(3) With increasing concentrations of the nutrient solution it was found in these experiments that the composition, expressed in percentages, and total quantity of N and K per plant were decidedly increased in the tops. This was also true for the roots, but in addition these showed a marked increase in the percentage and total quantity per plant of Ca and PO_4. In the tops most of the Ca, Mg, PO_4, and K was present in a water-soluble form. In the roots grown in the solutions of the higher concentrations large percentages of insoluble Ca and PO_4 were found.

(4) When plants of uniform development were transferred to nutrient solutions of different concentrations, a greater transpiration took place from solutions of low concentration. Absorption and transpiration took place independently, so that the solution under some circumstances might become either more or less concentrated, depending upon the original concentration of the solution.

(5) The optimum total concentration of the nutrient solution, if defined as the least concentration giving a yield equal to any higher concentration, was not found to be greater than that represented by 0.6 atmosphere osmotic pressure; and it may be less than 0.1 atmosphere. For the solutions used in these experiments, inhibitive concentrations were not higher than those represented by 2 to 2.5 atmospheres osmotic pressure.

(6) In the interpretation ofthe results of solution- and sand-culture experiments in terms of concentration and ionic ratios, emphasis is placed on the necessity of clearly distinguishing between the concentration and composition of the solution used and the total supply of the various elements provided for each plant. In the periods between changes of solution the concentration and composition may be markedly altered because of absorption by the plant. In many experiments the number of plants and size of culture vessels have been chosen arbitrarily without reference to these facts.

(7) From a consideration of previous experiments it is concluded that there is no sufficient evidence to prove that the plant requires for optimum yield any very specific ratio of ions or elements within wide limits, provided the total supply and concentration of essential elements are adequate.

(8) In solutions with an acid reaction (P_H 5 to 5.5) the absorption of several ions was greater than from a neutral solution (P_H 6.8) of similar composition and the same total concentration.

(9) One experiment suggested the possibility that at certain periods of growth excretion of electrolytes may take place, and that this phenomenon is dependent on the reaction and concentration of the nutrient solution.

(10) An acid reaction represented by P_H 5 was not found to be injurious to the barley plant at any period. There was a tendency toward the production of a neutral reaction in the solution as a result of changes in the equilibria due to absorption.

(11) When the plant was placed in a very dilute nutrient solution, excretion of electrolytes, especially Ca, Mg, PO_4, took place at first. This was followed by absorption which continued until the solution had a resistance comparable to that of the distilled water used in making the solutions.

(12) There was no evidence that the sand used significantly altered the concentration or composition of the nutrient solution. Other reasons are suggested for differences between sand and solution cultures.

(13) The mode of expressing the composition and concentration of the nutrient solution is discussed, and it is suggested that the theoretical total osmotic pressure of the nutrient solution is not necessarily significant in its relation to the plant. It is also concluded that the interpretation of results should be based on the composition of the solution in terms of ions or radicles rather than of the salts used in preparing the solution.

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NATURAL CARBONATES OF CALCIUM AND MAGNESIUM IN RELATION TO THE CHEMICAL COMPOSITION, BACTERIAL CONTENTS, AND CROP-PRODUCING POWER OF TWO VERY ACID SOILS

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INTRODUCTION

Agricultural limestones so frequently contain large quantities of magnesium that the question of the relative values of calcium and magnesium carbonates as neutralizers of soil acidity is of great practical importance. Many instances where magnesium has had detrimental effects on plant growth have been reported, as have also other instances where magnesium limestones have produced greater crop growth than did pure calcium limestones. The literature on the subject is very extensive and has been fully reviewed by others (*3, 4, 5, 6*).[2]

The present paper reports results of pot and laboratory tests on two very acid soils of distinctly different types. The data were all obtained from experiments conducted under controlled moisture conditions with natural carbonates of high purity. The calcite used analyzed 56 per cent calcium oxid and 0.1 per cent magnesium oxid. The dolomite contained 30.4 per cent calcium oxid and 20.5 per cent magnesium oxid. The magnesite contained 0.12 per cent calcium oxid and 46.2 per cent magnesium oxid. No basic or artificial carbonates were used in the experiments reported.

SOILS USED

The analyses of the soils used, an acid silty clay very low in organic matter content and an acid black peaty sand high in organic matter content, are given in Table I.

The two soils are shown to be of quite different composition. The variation between the nitrogen and humus determinations is large. Both the Hopkins [3] potassium-nitrate and the Jones [4] calcium-acetate

[1] Resigned Nov. 1, 1918.

[2] Reference is made by number (italic) to "Literature cited," p. 125.

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method show the soils as quite acid. That the substances in the soil which react with the potassium nitrate and calcium acetate are not entirely the same is plainly shown in the different acidity results obtained by the two methods. Neither soil can be said to contain a normal amount of calcium, and the magnesium of both is more than twice the calcium content.

TABLE I.—*Analyses of soils used*

Determinations.[a]	Yellow clay.	Black sand.
	Per cent.	*Per cent.*
Volatile matter	3.57	10.13
Potassium oxid (K_2O)	.27	.21
Calcium oxid (CaO)	.18	.10
Magnesium oxid (MgO)	.40	.23
Manganese oxid (Mn_3O_4)	.08	.04
Ferric oxid (Fe_2O_3)	3.68	1.04
Aluminum oxid (Al_2O_3)	4.68	3.09
Phosphorus oxid (P_2O_5)	.05	.10
Sulphate (SO_3)	.12	.11
Residue	87.76	85.50
Nitrogen	.07	.40
Humus (acid)[b]	.73	5.72
Humus[c]	.70	4.96
Hygroscopic moisture	1.50	1.90
Acidity:	*Pounds.*[d]	*Pounds.*[d]
Potassium-nitrate method	2,730.00	1,260.00
Calcium-acetate method	2,940.00	5,310.00
	Gm.[e]	*Gm.*[e]
Water-holding capacity	48.6	67.1

[a] WILEY, H. W. OP. CIT.
[b] Ammonia soluble without previous washing with dilute hydrochloric acid.
[c] Washed with hydrochloric acid, digested with ammonia, filtered, and refiltered till clear.
[d] Pounds of calcium carbonate required to neutralize one million pounds of soil.
[e] Grams of water per 100 gm. of dry soil.

GREENHOUSE TESTS

Pot tests were conducted in duplicate for all treatments on each soil. The crops in the order in which they were grown were wheat, red clover, and blood turnip beets. The containers for the soil were galvanized iron pots, 9.25 inches in diameter and 11 inches deep, paraffined well on the inside. One-inch galvanized iron tubes, connected with an arch at the bottom of the pot (both well paraffined), were provided for aeration and the addition of water. The seed was selected with care, planted uniformly, and the resulting plants were kept under good conditions for their development. The soil was kept at optimum moisture content by weighing the pots at regular intervals and replenishing the water lost with distilled water. The various soil treatments employed and the crop yields are given in Table II.

Table II shows that the 4,000-pound applications of calcite, magnesite, and dolomite gave similar results on both soils. The differences between the calcite, magnesite, and dolomite increases were small for the wheat and clover, while the magnesium carbonate gave much larger increases with the beets.

TABLE II.—*Yields of air-dry wheat and clover and undried blood turnip beets on acid soils treated with natural carbonates of calcium and magnesium*

Pot No.	Treatment per million pounds of soil.[a]	Average yields in grams per pot.							
		Yellow clay.				Black sand.			
		Wheat.	Clover.	Beets.		Wheat.	Clover.	Beets.	
				Tops.	Roots.			Tops.	Roots.
3	Control.....	44.0	2.0	0.0	0.0	1.5	3.5	0.0	0.0
5	4,000 pounds calcite....	65.5	18.5	36.0	18.0	31.5	12.5	23.0	13.5
10	4,000 pounds magnesite..	64.0	16.0	53.0	33.5	29.0	8.5	51.0	23.0
9	4,000 pounds dolomite...	62.5	20.0	44.5	22.5	35.5	11.5	33.0	21.0
13	12,000 pounds calcite...	77.5	15.5	56.5	49.5	51.0	15.0	50.0	21.0
14	12,000 pounds magnesite	71.0	16.5	63.0	51.5	.0	.0	3.0	.5

[a] A basic application of 72 pounds diammonium phosphate and 100 pounds dipotassium phosphate per million pounds of soil was made to each pot. In addition the yellow clay soil received a total of 90 pounds per million of ammonium nitrate, applied in three equal successive applications.

The 12,000-pound applications of calcite and magnesite gave different results on the two soils. The most apparent of these differences were noted in the detrimental effects of the 12,000-pound magnesite application on the black sand and the increased crop yields on the yellow clay due to additional magnesite. While the increases due to heavier applications were not proportionally larger, they checked the results of the 4,000-pound applications. The greatest increases in crop yields for the 12,000- over the 4,000-pound applications of natural carbonates were with the calcite on the black sand. It should be noted that the yield of beets on the black sand, although much increased over that obtained with the 4,000-pound calcite application, is not so large as that obtained with the 4,000-pound treatment of magnesite.

These varying yields with different crops are in accord with the results obtained by Coupin (*1*) who found that the action of magnesium carbonate was different on different species of plants.

Plate 1 shows the appearance of the various crops grown on the acid yellow clay soil. Pot 4, not discussed in the tables, had an application of one-half as much calcite as pot 5.

Plate 2 shows the appearance of the various crops grown on the acid black sand. Pot 4 had one-half as much calcite as was applied to pot 5. The appearance of the beets in this series would indicate that magnesium was not so harmful to beets as it was to wheat and clover.

At certain stages of growth the wheat growing in the pots treated with magnesite showed a dark green color, as noted by others (*5*), while the wheat in the calcite-treated pots was a light yellowish green. Although the magnesite caused almost as much wheat increase in all except the 12,000-pound magnesite application on the black sand,

there was at times during the vegetative stage of growth a tendency toward tip burning wherever magnesite was applied. This unfavorable condition, somewhat similar to that observed by Dickson (*2*), was not noted on the calcite- or dolomite-treated wheat and did not appear to cause permanent injury in any case.

TABLE III.—*Soluble salts, nitrates, bacterial contents, acidity, and carbon-dioxid content of two acid soils as affected by natural carbonates of calcium and magnesium*

Treatment per million pounds of soil.	Soluble salts, parts per million.	Nitrate (NO_3) content, parts per million.		Percentage of carbon dioxid.	Acidity, as calcium carbonate needed per million pounds of soil.		Bacterial content, millions per gram of soil.		Ratio of calcium oxid to magnesium oxid.
		As sampled.	After nitrification.		Hopkins method.	Jones method.	Aerobic.	Anaerobic.	
YELLOW CLAY									
Control	[a]192	Trace.	Trace.	0.02	5,600	8,250	3.027	0.000	1:2.2
4,000 pounds calcite	272	0	184	.04	40	1,500	7.605	.000	1:1.0
4,000 pounds magnesite	604	0	365	.04	40	1,250	12.229	1.220	1:3.0
4,000 pounds dolomite	[b]...	.....	.....	.04	80	1,500	.....	.....	1:1.6
12,000 pounds calcite	424	0	873	.28	0	1,100	5.244	.000	2:1.0
12,000 pounds magnesite	624	0	842	.25	0	750	9.158	1.133	1:5.3
BLACK SAND									
Control	448	350	340	0.03	3,520	13,500	2.813	2.907	1.0:2.3
4,000 pounds calcite	280	52	913	.11	80	6,000	10.583	.099	1.4:1.0
4,000 pounds magnesite	360	220	1,000	.10	120	5,000	6.833	.420	1.0:4.0
4,000 pounds dolomite	.....	.....	.....	.10	160	5,500	.....	.....	1.9:1.8
12,000 pounds calcite	432	233	1,280	.23	20	1,500	16.037	1.760	3.4:1.0
12,000 pounds magnesite	1,320	915	2,544	.23	20	1,000	5.837	.625	1.0:7.8

[a] Figures calculated to dry basis.
[b] Blanks in dolomite column indicate that no determinations were made.

Table III gives the results of tests on the soils made 10 months after the experiments were started, when the pots contained growing clover and wheat stubble. The samples were drawn with Noyes' bacteriologists' soil samplers [1] to the full depth of the pots. Nitrates, bacterial numbers, nitrification and soluble salts were determined on the moist samples. Acidity and carbon dioxid were determined on air-dry samples.

Soluble salts were determined by means of the electrical bridge [2] and show the relative quantities of ionized salts in the soil solution. The nitrates were determined by the modified phenol-disulphonic acid method.[3] Nitrification was carried out in tumblers by the beaker method. Carbon dioxid was determined with boiling hydrochloric acid (specfic gravity 1.115). Acidity was determined by the Hopkins [4]

[1] NOYES, H. A. SOIL SAMPLING FOR BACTERIOLOGICAL ANALYSIS. *In* Jour. Amer. Soc. Agron., v. 7, no. 5, p. 239–249, fig. 13, pl. 4. 1915.

[2] DAVIS, R. O. E., and BRYAN, H. THE ELECTRIC BRIDGE FOR THE DETERMINATION OF SOLUBLE SALTS IN SOILS. U. S. Dept. Agr. Bur. Soils Bul. 61, 36 p., 7 fig., 2 pl. 1910.

[3] NOYES, H. A. ACCURATE DETERMINATION OF SOIL NITRATES BY PHENOL DISULFONIC ACID METHOD. *In* Jour. Indus. and Engin. Chem., v. 11, no. 3, p. 213–218. 1919.

[4] WILEY, H. W. OP CIT.

potassium-nitrate and by the Jones[1] calcium-acetate methods. Bacterial counts were the average of counts on five plates made after 10 days' incubation at 20° C. by the method of Noyes and Voigt.[2]

The quantities of salts, as determined by the electric bridge, were greater with the use of the magnesite than with the calcite. This, to a certain extent, illustrates the comparative solubilities of the calcium and magnesium compounds as well as of other soluble salts resulting from the reactions taking place between these carbonates and the soil constituents.

The carbon-dioxid determinations show that the decomposition of the added natural carbonates was not complete in any case at the end of 10 months. The data tend to confirm, however, MacIntire's statement (6) that magnesium carbonates are more readily decomposed than is calcium carbonate.

While each soil (Table I) contained approximately two parts of magnesium oxid to one part of calcium oxid there was almost twice as much of both calcium and magnesium oxids in the clay soil as in the sandy soil. Six tons of magnesite per million pounds of soil increased the magnesium oxid content by 0.57 per cent. This made the ratios of calcium oxid to magnesium oxid approximately 1 to 8 for the sandy soil and 1 to 5 for the clay soil. The 2-ton application of magnesite which was not injurious made the ratio of calcium oxid to magnesium oxid 1 to 4 for the sandy soil. It might be contended that the sandy soil would have produced crops with a ratio of 1 to 5 between calcium oxid and magnesium oxid, but it must be remembered that in any case only small portions of the total calcium and magnesium were in solution, and it is quite probable that the clay soil would offer more resistance to the injurious action of the magnesium salts than the sand would.

The 12,000-pound application of magnesite gave 1,320 pounds of soluble salts per million of the black sand and only 624 on the yellow clay. Recent tests by one of the writers (unpublished data) have shown that magnesium carbonate increases the solubility of soil constituents more than calcium carbonate does. Soluble magnesium in quantity has long been known to be detrimental to plant and bacterial development. This is in accord with both the high soluble salt content and low aerobic bacteria counts on the black sandy soil with the 12,000-pound application of magnesite.

In a previous paper (7) it has been shown that nitrification occurs in these acid soils and that the amounts of nitrates found in the soils when sampled are largely influenced by the growing crop. The nitrates after incubation are evidence that liming increases the nitrifying power of the soils. In all cases magnesite caused greater nitrification than did

[1] JONES, C. H. OP. CIT.

[2] NOYES, H. A., and VOIGHT, Edwin. A TECHNIC FOR THE BACTERIOLOGICAL EXAMINATION OF SOILS. *In* Proc. Ind. Acad. Sci. 1916, p. 272–301, 6 fig. 1917.

calcite. The number of aerobic organisms was increased by liming, which is evidence that in general those conditions that are favorable to plant growth are also favorable to bacterial activity.

On the yellow clay the magnesite caused greater bacterial growth, while on the black sand the calcite caused the greater increases. The bacterial differences between the magnesite and calcite results on these two soils are probably partly due to the relative availability of the plant food present. The magnesite, which increases soluble soil constituents more than calcite, gave the greater increases on the yellow clay—low in available plant food—and the lesser increases on the black sand—relatively richer in available plant food. The heavy application of magnesite unhindered by clay, with which it would form insoluble compounds, caused too high a concentration of soluble salts on the black sand. The black sand, though less compact, was high enough in organic matter to contain normally the more anaerobes.

The crop results do not point to any particular ratio between calcium and magnesium which could be called optimum for either soil or crop. This is in accord with the results of Waynick (*8*) and others.

It is not possible with the data at hand to determine how much the injurious action of the high magnesite application on the black sand was due to an unfavorable calcium-magnesium ratio and how much was due to the high concentration of soluble magnesium salts; but, in view of the fact that the black sand soil still gave an acid reaction after the heaviest magnesite application, it is evident that the crop injury was not due to alkalinity.

SUMMARY

(1) Pot experiments were conducted on two very acid soils of different types, using the natural carbonates, calcite, dolomite, and magnesite, in varying amounts. Wheat, red clover, and blood turnip beets were grown in succession.

(2) After being cropped 10 months under optimum moisture conditions the soils were tested for soluble salts, nitrates, nitrification, carbon dioxid, acidity, and both aerobic and anaerobic bacteria.

(3) Although both soils originally contained twice as much magnesium oxid as calcium oxid, still calcite, dolomite, and magnesite, in both quantities used, produced, with one exception, good crop increases on both soils. The 6-ton application of magnesite on the black sand soil killed the crops.

(4) Good crop increases were obtained with carbonate applications which produced ratios of calcium oxid to magnesium oxid varying from 2:1 to 1:5.3 on the yellow clay soil and from 3.4:1 to 1:4 on the black sand. The 6-ton application on the black sand which caused crop failure gave a ratio of 1 calcium oxid to 7.9 magnesium oxid.

(5) Wheat, red clover, and beets responded differently toward calcium and magnesium carbonates. With the medium applications beets were

benefited more by magnesium carbonate, while wheat and clover gave greater increases with calcium than with magnesium carbonate.

(6) Magnesite in all instances increased the concentrations of soluble salts in the soils more than calcite.

(7) Carbon dioxid determinations snowed that the carbonates were not entirely decomposed at the end of one year. The decomposition of the magnesite seems to have proceeded faster than that of the calcite.

(8) Magnesite produced more favorable conditions for nitrification than did calcite.

(9) Magnesite encouraged the multiplication of both aerobic and anaerobic bacteria on the yellow clay soil more than calcite did. On the black sand soil the reverse was true. Calcite increased the bacterial content of the soil more than did magnesite.

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PLATE 1

Pot cultures on acid yellow clay soil:
A.—Wheat; B.—Red clover; C.—Red turnip beets.

SERIES NO.	TREATMENT PER MILLION POUNDS SOIL.
D 3	No carbonates.
D 4	1 ton calcite.
D 5	2 tons calcite.
D 9	2 tons dolomite.
D 10	2 tons magnesite.
D 13	6 tons calcite.
D 14	6 tons magnesite.

A
D 3
D 4
D 5
D 7
D 10
D 13
D 14
B
D 3
D 4
D 5
D 7
D 10
D 13
D 14
C
D 3
D 4
D 5
D 9
D 10
D 13
D 14

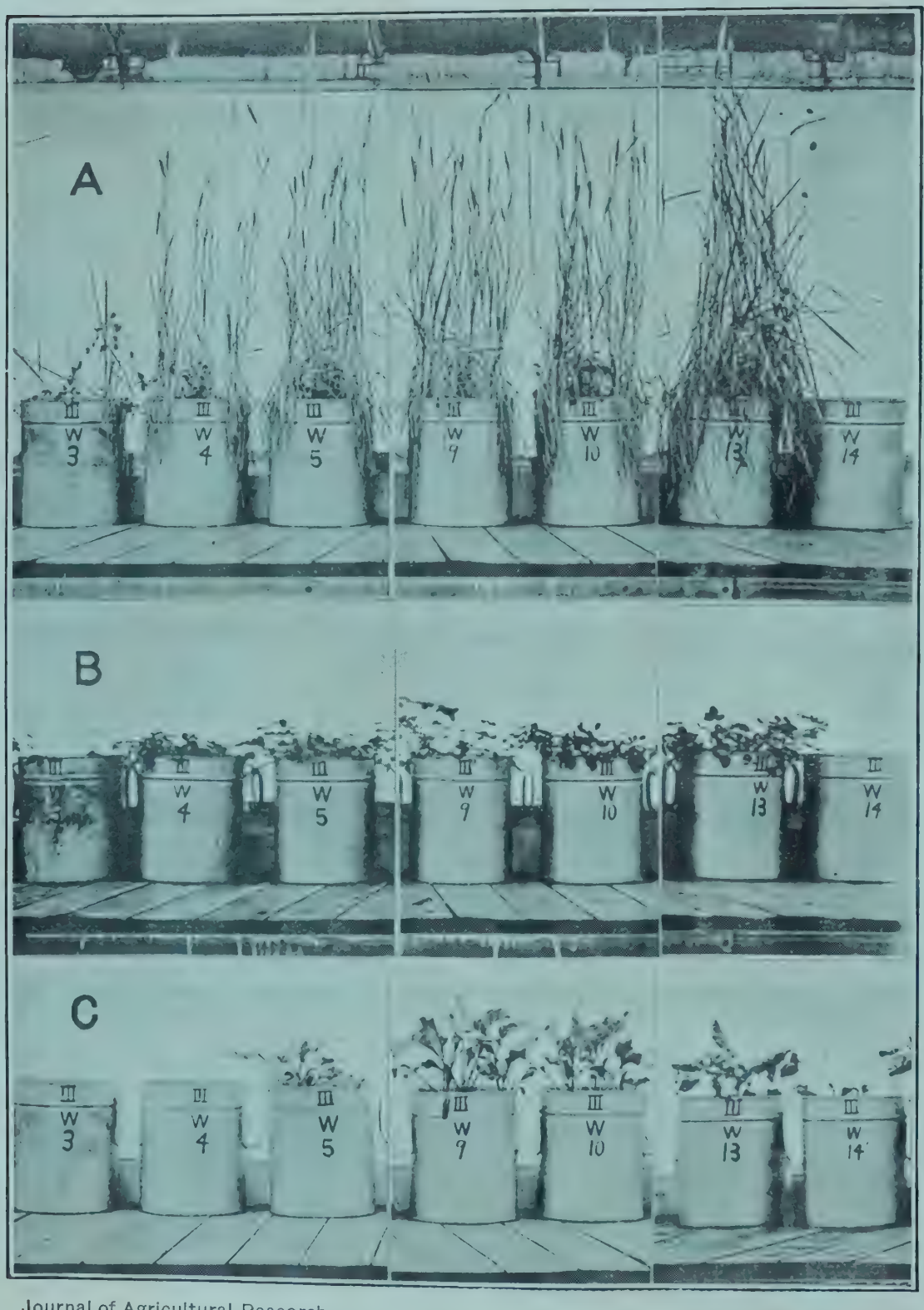
A
W 3
W 4
W 5
W 9
W 10
W 13
W 14
B
W 4
W 5
W 9
W 10
W 13
W 14
C
W 3
W 4
W 5
W 9
W 10
W 13
W 14

PLATE 2

Pot cultures on acid black sand soil:
A.—Wheat; B.—Red clover; C.—Red turnip beets.

SERIES NO.	TREATMENT PER MILLION POUNDS SOIL.
W 3	No carbonates.
W 4	1 ton calcite.
W 5	2 tons calcite.
W 9	2 tons dolomite.
W 10	2 tons magnesite.
W 13	6 tons calcite.
W 14	6 tons magnesite.

RECENT STUDIES ON SCLEROTIUM ROLFSII SACC.

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ECONOMIC IMPORTANCE OF THE FUNGUS

As a parasite *Sclerotium rolfsii* Sacc. is of the same economic importance to the South as *Sclerotinia libertiana* Fck. is to some of the more northern States. Rolfs (*12*, *13*, *14*)[1], in Florida, and Earle (*1*), in Alabama, found it to be serious on tomatoes. In Louisiana, Edgerton and Moreland (*3*, *p. 19*) also found it upon tomatoes. Fulton (*4*, *p. 3*), states that it often ruins the pepper crop in Louisiana. Wolf (*2*, *p. 142–146*), in Alabama, and McClintock (*10*), in Virginia, have fully recognized its economic importance as the causal organism of a peanut trouble. Earle and Rogers (*2*) and Wolf (*21*) have studied a citrus disease due to *Sclerotium rolfsii*, and Peltier (*11*) has also found it on cultivated perennials. Godfrey (*5*) found it on wheat. In Texas, the writer observed *Sclerotium rolfsii* attacking cantaloupes, tomatoes, peppers, peanuts, watermelons, young cotton seedlings, sweet potatoes, radish, cabbage, young corn plants, Bermuda grass, and a large number of weeds. It is to be regretted that there are no definite statistical data on crop losses from the attacks of this fungus. However, a conservative estimate might place these losses at about 5 per cent of the southern crops. In Texas *Sclerotium rolfsii*, although widely distributed, seems restricted to the sandy or sandy loam soils, the greatest damage occurring in wet seasons. The writer has repeatedly failed to find it on the heavy waxy soils where *Ozonium omnivorum* Sh. is so prevalent. The fungus is an air-loving organism, so it commonly finds an ideal environment in the light sandy soils.

Sclerotium rolfsii, although apparently occurring only in the South, has been recently reported by Peltier (*11*) for the first time in Illinois. It seems rather difficult to account for its sudden appearance there. Halsted (*6*,*7*), in working with a pure culture of this fungus originally derived from Florida, found considerable difficulty in obtaining positive infection with plants in New Jersey. The fungus has, so far as known, not been found there as a field trouble. The writer had difficulty in infecting healthy plants in Delaware, in 1912, with a pure culture sent to him from Alabama. It is to be remembered, too, that in Delaware *S. rolfsii* does not occur as a field parasite. It is probable that in Illinois the fungus was introduced with imported ornamentals from the South. In

[1] Reference is made by number (italic) to "Literature cited," p. 37–138.

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that case, and as explained by Peltier (*11*), the extremely wet summer in 1915 at Urbana, Ill., together with an average low temperature of 74° F. may account for the sudden appearance of the disease. *S. rolfsii* may not be able to withstand the cold winters of Illinois, in which case there should be no fear of its further spread. However, should it withstand the cold winters of Illinois, it would be reasonable to suppose that it is adapted to colder climates, and that if not guarded against may spread to other Northern States. Failure by Halsted and by the writer to infect plants in New Jersey and in Delaware may be explained by the fact that both worked with strains grown too long on artificial media. This, however, needs further verification.

HOST PLANTS

Sclerotium rolfsii is known to attack a large number of various genera and species of plants. Table I shows the wide adaptability of this fungus to different hosts.

TABLE I.—*Hosts affected by Sclerotium rolfsii*

Host.	Authority.	Year.	State.
Arachis hypogaea	Earle, F. S.	1900	Alabama.
	Rolfs, P. H.	1896	Florida.
	McClintock, J. A.	1917	Virginia.
	Taubenhaus, J. J.	1917, 1918	Texas.
Beta vulgaris	Earle, F. S.	1900	Alabama.
	Rolfs, P. H.	1896	Florida.
Brassica oleracea	....do	1896	Do.
	Taubenhaus, J. J.	1916, 1918	Texas.
Capsicum annum	Fulton, H. R.	1908	Louisiana.
	Taubenhaus, J. J.	1917, 1918	Texas.
Cucurbita spp.	Rolfs, P. H.	1896	Florida.
	Taubenhaus, J. J.	1918	Texas.
Citrullus vulgaris	Rolfs, P. H.	1896	Florida.
	Taubenhaus, J. J.	1916, 1917, 1918	Texas.
Chrysanthemum spp.	Rolfs, P. H.	1896	Florida.
Campanula spp.	Peltier, G. L.	1916	Illinois.
Colocasia esculenta	Harter, L. L.	1916	
Daphne spp.	Rolfs, P. H.	1896	Florida.
Dianthus plumarius	Peltier, G. L.	1916	Illinois.
Dracocephalum argenese	....do	1916	Do.
Edgeworthia papyrifera	Fulton, H. R.	1918	Louisiana.
Epilobium angustifolium	Rolfs, P. H.	1896	Florida.
Erigeron spp.	....do	1896	Do.
Expatorium ageratoides	Peltier, G. L.	1916	Illinois.
Ficus carica	Rolfs, P. H.	1896	Florida.
Fragaria spp.	Earle, F. S.	1900	Alabama.
Gossypium hirsutum	Rolfs, P. H.	1896	Florida.
	Taubenhaus, J. J.	1917, 1918	Texas.
Hydrangea pani	Rolfs, P. H.	1896	Florida.
Ipomoea purpurea	....do	1896	Do.
	Taubenhaus, J. J.	1917	
Lycopersicum esculentum	Rolfs, P. H.	1893, 1898	Do.
	Earle, F. S.	1900	Alabama.
	Taubenhaus, J. J.	1916, 1917, 1918	Texas.
Phaseolus vulgaris	Earle, F. S.	1900	Alabama.
	Fulton, H. R.	1908	Louisiana.
	Rolfs, P. H.	1896	Florida.

TABLE I.—*Hosts affected by Sclerotium rolfsii*—Continued

Host.	Authority.	Year.	State.
Penstemon spp.	Peltier, G. L.	1916	Illinois.
Phlox sublata	do	1916	Do.
Rheum rhaponticum	Rolfs, P. H.	1896	Florida.
	Taubenhaus, J. J.	1918	Texas.
Solanum tuberosum	Earle, F. S.	1900	Alabama.
	Rolfs, P. H.	1896	Florida.
	Taubenhaus, J. J.	1917	Texas.
Saccharum officinarum	Fulton, H. R.	1908	Louisiana.
	Wakker, J. H.	1897	Java.
Solanum melongena	Rolfs, P. H.	1896	Florida.
	Taubenhaus, J. J.	1918	Texas.
Vigna sinensis	Earle, F. S.	1900	Alabama.
	Rolfs, P. H.	1896	Florida.
	Taubenhaus, J. J.	1917, 1918	Texas.
Viola odorata	Rolfs, P. H.	1896	Florida.
	Taubenhaus, J. J.	1918	Texas.
	Harter, L. L.	1916	Do.
Xanthosoma sagitifolium	do	1916	Do.

Though *Sclerotium rolfsii* attacks a large number of hosts, its virulence is more pronounced on tender plants and growth. As a storage-rot its economic importance can not be overlooked. Pumpkins, squashes, cabbage, and Irish and sweet potatoes are often seriously affected under storage conditions when the necessary ventilation is lacking.

NAME OF THE DISEASE

Plant pathologists are aware of the necessity of standardizing names of plant diseases. The disease here considered seems to have as yet no standard name. Rolfs (*12*) and Fulton (*4*) named it "blight," and Earle (*1*) and Edgerton (*3 p. 19*) called it "Sclerotium wilt." McClintock (*10*) terms it "wilt," and Wolf (*20, p. 142–146*), working on a peanut disease, named it "Sclerotial rot." Peltier (*11*) does not give it any particular name, but merely refers to it as a "disease" or "rot." Stevens and Hall (*18, p. 259–262*) and the writer (*19, p. 305*) have referred to it as "southern blight." However, this term is misleading, since the bacterial blight of tomatoes named by Halsted (*6*), in Mississippi, as southern blight is generally accepted now as being caused by *Bacillus solonacearum* E. F. Sm. Neither would the term "Sclerotium rot" or "wilt" be tenable, since there are several species of Sclerotium fungi known to produce disease in plants. The term "southern Sclerotium rot" is therefore proposed. This will suggest the nature of the causal organism and its southern origin.

PATHOGENICITY AND RACIAL STRAINS

Few of the writers on *Sclerotium rolfsii* have mentioned an attempt to carry out pure culture inoculations to prove the pathogenicity of the fungus. It seems to have been taken for granted that this fungus is a

parasite. Of those who report such attempts Wolf (*20*) may be mentioned. He inoculated various legume seeds by stirring in a small quantity of water containing a macerated vigorous culture of *S. rolfsii.* The inoculated seed was then planted in the greenhouse, in a soil which was previously treated with formaldehyde. As a result of this treatment positive infections were obtained with some of the legumes used. Positive infections with *S. rolfsii* on pepper plants were also recorded by Fulton, (*4, p. 3–8*).

In order to establish definitely the pathogenicity of *Sclerotium roflsii*, and also to determine whether or not there existed varietal or physiological strains in this organism, the inoculations reported in Table II were carried out. All hosts were covered with bell jars for 24 hours immediately after inoculation. All inoculations were performed in the greenhouse.

Although many more inoculations have been made than are reported in Table II, these will show that *Schlerotium rolfsii* is an active parasite.

A study of Table II shows further that there are no varietal or physiological strains in this organism. A strain isolated from tomatoes, for instance, will infect a large number of other hosts. This is also true when a strain from a cantaloupe is isolated. Table II also shows that no infection whatsoever could be obtained with a test tube culture 1 year old, even though the same was mixed with sterilized soil where it had no competition with any other of the soil floras and where it had plenty of available food. However, just as soon as a transfer was made from the 1-year-old culture it revived and assumed its normal virulence. It was then in no way different from a fresh strain of *Sclerotium rolfsii* recently isolated from a normally infected plant in the field.

Sclerotium rolfsii is truly parasitic, since it is not always necessary to produce infection through puncture inoculations. As shown in Table II, positive infections were obtained when a pure culture of the fungus was merely worked into the soil where moisture was present. These same observations were also reported by Fulton (*4*). However, Harter (*8, 9*) could not obtain any infection on *Colocasia esculenta* or *Xanthosoma sagitifolium* unless inoculations were made by means of a puncture. The writer has had similar experiences in his inoculations with the Irish and sweet potatoes (Pl. 5, B, C) as well as the orange and the apple. In these cases infections were possible only through a wound. On the other hand, however, as soon as infection took and the rot had progressed sufficiently all that was necessary then to infect a healthy tuber of Irish potatoes was to place the latter in close proximity to the former and infection would result without the aid of a puncture. In this case, apparently, the fungus assumed added vigor on the first inoculated tuber and was therefore capable of penetrating the other without the aid of a wound. In Colocasia and Xanthosoma the corms are protected by hard scales, and infection becomes possible only by means of a wound.

TABLE II.—*Inoculation with pure cultures of Sclerotium rolfsii on various hosts*

Source and age of culture.	Date of inoculation.	Host inoculated.	Method.	Result.	Controls.
1a. Delaware strain, 1 year old[a]	July 9, 1917	15 tomato plants, 6 weeks old.	Fungus worked into sterile soils.[b]	All healthy.....	5, all healthy.
Do.....	.....do.....	10 peanut plants.	.....do.....	.....do.....	Do.
Do.....	.....do.....	3 cabbage heads.	Puncture.....	.....do.....	2, both healthy.
Do.....	.....do.....	10 tubers Irish potatoes.	.....do.....	.....do.....	5, all healthy.
1b. Transfer from 1a, 10 days old.	.....do.....	15 tomato plants, 6 weeks old.	Fungus worked into sterile soil.	12 positive infections.	Do.
Do.....	.....do.....	10 peanut plants.	.....do.....	6 positive infections.	Do.
Do.....	.....do.....	3 cabbage heads..	Puncture.....	All positive infections.	2, 1 healthy, 1 black-rotted[c]
Do.....	.....do.....	10 tubers Irish potatoes.	.....do.....	.....do.....	5, all healthy.
1c. Fresh strain, isolated from cantaloupe, 10 days old.	July 15, 1917	6 tomato plants, 5 weeks old.	Fungus worked into soil.	5 positive infections.	Do.
Do.....	.....do.....	14 sweet-potato plants.	.....do.....	9 positive infections.	3, all healthy.
Do.....	.....d9.....	2 watermelon fruits.	Puncture.....	Both positive infections.	2, both healthy.
Do.....	.....do.....	20 sweet-potato roots.	.....do.....	17 positive, 3 soft rotted from Rhizopus.	6, all healthy.
Do.....	.....do.....	10 cantaloupe fruits.	.....do.....	All positive infections.	2, both healthy.
Do.....	.....do.....	6 gourd fruits...	Puncture.....	.....do.....	Do.
1d. Fresh strain isolated from tomato in field, 8 days old.	July 16, 1917	8 tomato plants, 12 weeks old.	Fungus worked into soil.	5 positive infections.	Do.
Do.....	.....do.....	3 peanut plants, 9 weeks old.	.....do.....	All positive infections.	Do.
Do.....	.....do.....	10 sweet-potato plants.	.....do.....	9 positive infections.	5, all healthy.
Do.....	.....do.....	20 corn plants, 3 weeks old.	.....do.....	8 positive infections.	10, all healthy.
Do.....	.....do.....	6 watermelon fruits.	Puncture.....	All positive infections.	2, both healthy.
Do.....	.....do.....	5 cantaloupe fruits.	.....do.....	.....do.....	Do.
Do.....	.....do.....	10 unripe banana fruits.	.....do.....	All positive infections, anthracnose present.	5, 4 healthy, 1 rotted from anthracnose.
Do.....	.....do.....	6 sweet-potato roots.	.....do.....	5 positive infections, 1 soft-rotted from Rhizopus.	3, all soft-rotted from Rhizopus.
Do.....	.....do.....	12 oranges.....	.....do.....	All positive infections.	4, all healthy.
Do.....	.....do.....	12 apples, variety unknown.	.....do.....	.....do.....	3, all healthy.
Do.....	.....do.....	5 squashes.....	.....do.....	.....do.....	2, both healthy.
Do.....	.....do.....	5 pepper plants, 7 weeks old.	Fungus worked into soil.	4 positive infections, 1 doubtful.	Do.
1e. Fresh strain isolated from peanut in field.	Aug. 19, 1917.	7 tomato plants, 5 weeks old.	.....do.....	5 positive infections.	Do.
Do.....	.....do.....	7 peanut plants, 5 weeks old.	.....do.....	All positive infections.	4, all healthy.
Do.....	.....do.....	12 sweet-potato plants, 4 weeks old.	.....do.....	.....do.....	5, all healthy.
Do.....	.....do.....	10 tubers Irish-potatoes	Puncture.....	8 positive infections.	4, all healthy.
Do.....	.....do.....	8 sweet-potato roots.	.....do.....	All positive infections.	5, all healthy.

[a] Culture originally obtained from Alabama.
[b] Fungus mucelium crushed up in sterile water, then worked in about ½ inch deep.
[c] Black-rotted by *Pseudomonas compestris* (Pam) Ew. Sm.

PERIOD OF INCUBATION

The findings of the writer substantiated those of others—namely, that with growing plants such as tomatoes, peanuts, corn, cotton seedlings, sweet potatoes, and others the period of incubation ranges from 2 to 4 days, depending on the tenderness of the growing tissue. Wilting usually begins after the second day, and the plant usually dies in from 4 to 6 days after inoculation. In inoculations of tubers of Irish potatoes, infection becomes apparent in about 6 days. After 15 days the potatoes are about half rotted (Pl. 5, B), the injury being a typical "melter," the tubers becoming very soft and at the least pressure rupturing and liberating a clear liquid with an agreeable odor of fermentation. With sweet potatoes the period of incubation is about the same as that of Irish potatoes, with the difference that the rot produced is not of the "melter" type. The infected tissue becomes browned, water-soaked at first but firm, then hard and stringy (Pl. 5, C). With the cantaloupe the period of incubation is usually from 3 to 6 days, after which the rot progresses very rapidly. If the inoculated melon is so placed that the point of infection touches the glass, the rot works so rapidly that it practically melts away half of the fruit, leaving a ragged cavity (Pl. 3, E). On the other hand, if after inoculation the fruit is so placed that the point of infection is turned upward and not allowed to touch the glass, the rot progresses slowly without producing a rapid soft rot. At the same time the fungus hyphae permeate the fruit (Pl. 3, F) and form a luxuriant growth which spreads all over the surface of the cantaloupe (Pl. 3, D). After the contents of the fruit are destroyed by the fungus, all that remains is a compacted mass of mycelium, which later rounds itself up into one mass of small sclerotia (Pl. 3, C). With the watermelon or the squash the period of incubation varies from 8 to 10 days, the inoculated fruit dry-rotting very slowly. The incubation period for the banana varies from 4 to 8 days, and for the orange and the apple from 6 to 10 days. Once infection starts these fruits rot very rapidly.

MODE OF INFECTION

It has already been pointed out that abrasion of the host is not necessary for infection. This is especially true with tender growing plants. Of paramount importance to infection may be considered moisture and especially air. For successful soil inoculations it is necessary to cover the fungus not more than ½ to 1 inch deep. Numerous laboratory experiments indicate that no infection is possible if either the mycelium or the sclerotia are buried more than 5 inches deep. This suggests that deep plowing would control the trouble. Furthermore, infection seems possible only where a young and actively growing culture is used.

Infection seems to be favored by an enzym secreted by the advancing mycelial strands. Examination of the roots or tubers infected with

Sclerotium rolfsii always shows a distinct zone of demarkation preceding the rotted area. Careful miscroscopic examination of the tissue in this zone does not show the presence of any fungus hyphae. Numerous attempts in culturing such tissue failed to produce any growth whatsoever. Moreover, the fungus always advances in large tufts or strands, which are composed of numerous hyphae joined together for the purpose, apparently, of secreting more enzyms to kill the host cells and to facilitate the more rapid progress of the rotting. In the large amount of embedded material which was sectioned and stained, no evidence was found to indicate that the growing tips of the advancing hyphae penetrate the cells of the host. Their purpose is apparently enzymic secretion. On the other hand, penetration seems always to be effected by the secondary hyphal branches which are formed at some distance below the growing tips. These usually penetrate the host through the stomata of the epidermis, then work inward; or they may break directly through the epidermal cells.

With starchy roots, such as those of sweet and Irish potatoes, the fungus apparently has difficulty in penetrating the cells which are gorged with starch. Studies and observations in this direction show that the enzym merely dissolves the middle lamella of the cells (fig. 1, H) and that the fungus hyphae are not found within the cells but only between them, where the middle lamella has disappeared. On the other hand, with soft tissue, especially with cantaloupes, the fungus hyphae are capable of piercing the cell walls and of working both within and between the cells (fig. 1, A, B, C). In migrating from one cell to another of the host tissue, the tip end of the mycelium attaches itself closely to the cell wall, then rounds up into a small ball, which develops a sharp point that pierces the cell wall. When this is accomplished the tip end again swells slightly, then straightens out, and grows in the usual way (fig. 1, B, C).

SYMPTOMS

The symptoms on actively growing plants are very striking. With the tomato, sweet potato, peanut, pepper, corn, as well as other tender plants, infection invariably starts at the foot of the plant from ½ to 1 inch below the ground level. Early infection is at first manifested by deep brown lesions. At this stage the host exhibits a slight wilting, as though suffering from a lack of water in the soil. Soon after, however, the lesions become covered with white radiating mycelium which encircles the foot of the plant. At this stage the epidermis and the cambium become water-soaked but remain firm, the foliage droops, loses its green color, and the plant never revives. The fungus seldom works to any considerable extent upward on the main stem; but it always works downward toward the main root and rootlets, especially those which are nearest to the surface. If a dead plant is pulled out, its roots and

rootlets are usually found covered with a white weft of the mycelium of the causal fungus. If the soil is kept moist and the dead plant remains untouched, the fungus will grow out on the surface of the soil in radiating

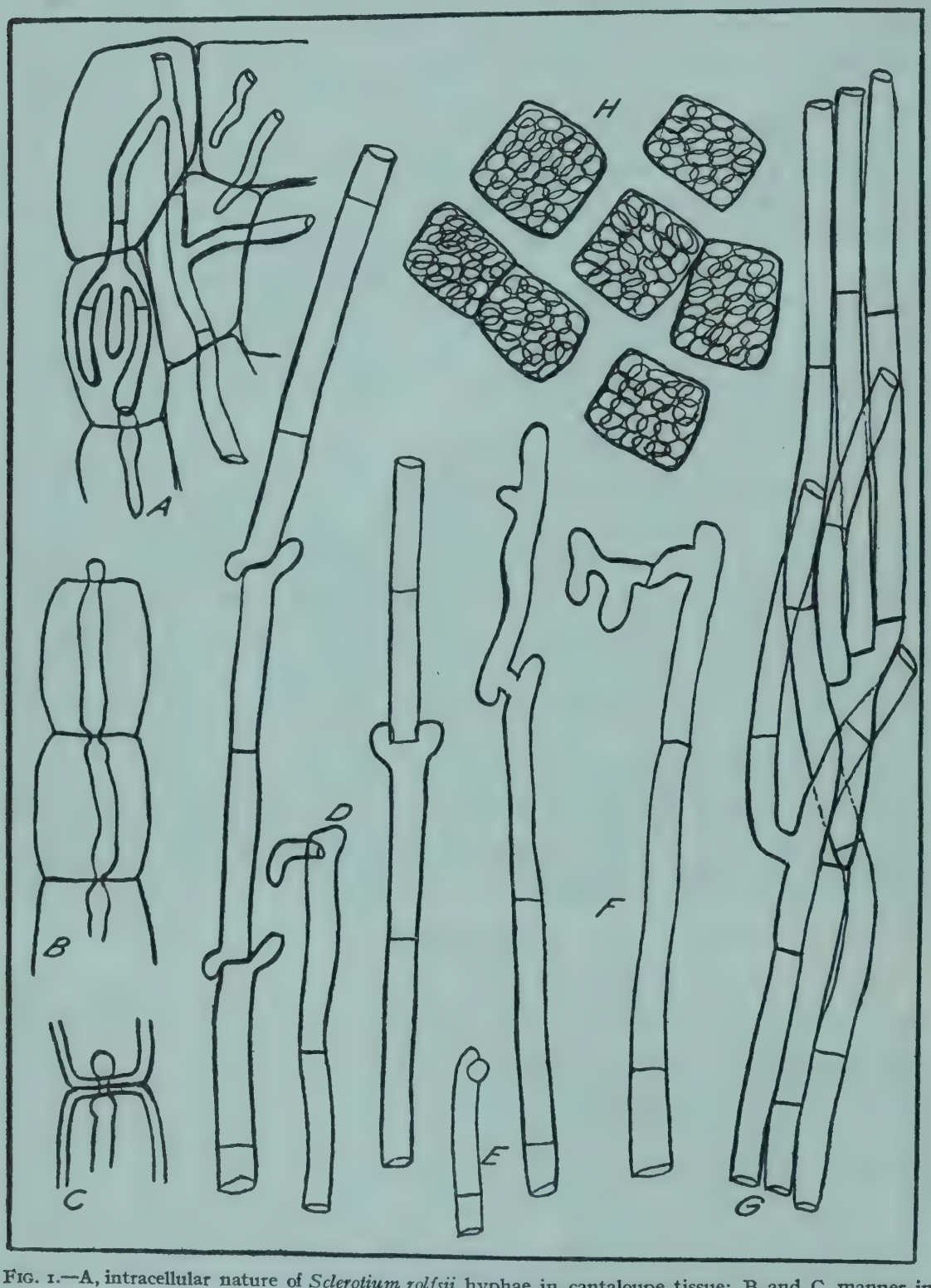

FIG. 1.—A, intracellular nature of *Sclerotium rolfsii* hyphae in cantaloupe tissue; B and C, manner in which the fungus pierces host cells; D, E, and F, method of budding and formation of new mycelial growth; G, manner of growth of mycelium, forming strands; H, dissolved middle lamellae of host cells.

fans around the foot of the dead plant (Pl. 6, A). With sweet potatoes in the seed bed the fungus often attacks young sprouts as soon as growth starts, in which case the mycelial strands work their way upward and

invade the entire tender stems (Pl. 5, A), which soften considerably and become covered with minute sclerotia. The fungus then works downward and rots the mother sweet potato. With stored cabbage the rot is confined to the two outermost layers of the head, which blacken and turn soft (Pl. 4, A).

MANNER OF GROWTH

The fungus was first studied by Halsted (*6*) and later by others, who, however, did not give it a specific name. Saccardo (*15*) named it *Sclerotium rolfsii* from specimens sent to him by Stevens (*17, p. 660–661*). In pure culture the growth of this organism is very distinct and can not easily be mistaken for any other species of Sclerotium fungus. Broadly speaking, this organism is little influenced by the kind of artificial medium on which it grows. Its myceleum is always white, fluffy, usually growing in strands and in radial fans (Pl. 4, B). This is especially true when an infected fruit is placed in a bell jar in contact with the glass. In a very short time the fungus grows out luxuriantly from the host on the surface of the glass, on which it forms beautiful radial fans (Pl. 6, B). The sclerotia, in pure cultures, are very little influenced as to size by the nature of the medium. In general they are of the size of a mustard seed (Pl. 4, B; 3, A). This, in fact, agrees with the general description of other workers. However, the sizeof the sclerotia is decidedly influenced by the kind of host which the causal organism infects. For instance, on cantaloupes and tomatoes the sclerotia are about the size of a mustard seed (Pl. 4, C). However, on the orange the sclerotia assume such large proportions (Pl. 3, B) as to resemble not those of *Sclerotium rolfsii* but rather those of *Sclerotinia libertinana* Fck. However, the writer had no trouble to plant these large sclerotia on artificial media and obtained again the normal growth of *Sclerotium rolfsii*, with its accompanying mustard-seed-like sclerotia. On the apple no sclerotia were formed at all. No experiments have been made to determine the effect of fruit acids on the size of the sclerotia of *Sclerotium rolfsii*, although it seems probable that the acid in the orange is responsible for the abnormally large development of the sclerotia. Peltier (*11*) has similarly observed that the sclerotia of *Sclerotium rolfsii* on cultivated perennials in Illinois were much larger in size than those found by the other workers. Similar observations are recorded by Smith (*16*). This would seem to indicate that the kind of host has an influence on the size of the sclerotia of this fungus.

One further peculiarity which the writer observed in the growth of the mycelium of this fungus is worthy of mention. Growth, instead of proceeding indefinitely at the terminal end of the original mycelial thread, comes to a standstill, a bud is developed near the tip end of the terminal cell, and only the bud continues growth (fig. 1, D–F). The hyphae are seldom found growing singly but always appear in groups of several branches (fig. 1, G), which often anastomize and form regular strands.

SEXUALITY

The observations of the writer lead him to believe in the existence of plus and minus strains in *Sclerotium rolfsii*, although they are only rarely met with. In June, 1917, an isolation of the casual fungus was made from damped-off cotton seedlings in the greenhouse. The infected tissue was first dipped for one second in a solution of 1 part corrosive sublimate in 2,000 parts of water, then carefully rinsed in sterilized water. It was then crushed with sterile forceps and mixed with melted and properly cooled medium, which was poured in a plate. After four days' growth in the Petri dish, sclerotia formed at the line where two colonies met (Pl. 4, D). This at once suggested a sexual act. Numerous transfers were made of the apparently different strains and were marked plus and minus. Whenever these strains were planted in the same Petri dish, sclerotia would always form at the line of union of colonies of the two strains. This was repeated many times with the same results. On the other hand, when each of these strains was planted separately few or no sclerotia would form. Moreover, when other varieties not supposed to have sexual strains were planted in the same Petri dish the sclerotia would form at random, and none would develop at the place of union of the two colonies (Pl. 3, A). Unfortunately, during a brief absence of the writer on emergency war work the plus and minus strains of *S. rolfsii*, together with many other cultures, were thrown out by a temporary employee in the laboratory.

SUMMARY

(1) *Sclerotium rolfsii* is prevalent throughout the Southern States. It has also been found recently in Illinois.

(2) The fungus attacks a large variety of cultivated crops in the field, ornamentals included. It also causes a serious trouble in stored vegetable product.

(3) The fungus is a true parasite, found mostly in the light sandy loams. Air and moisture are both necessary for infection. If buried too deep in the soil the organism apparently dies, hence deep ploughing is suggested as a control measure.

(4) There are no varietal nor physiological strains in *Schlerotium rolfsii*.

(5) The period of incubations varies from two to six days.

(6) The size of the sclerotia in pure cultures is little influenced by the medium used. It is, however, considerably influenced by the host. On the orange the sclerotia assume unusual proportions, resembling more the sclerotia of *Sclerotinia libertiana*.

(7) The mycelium of *Sclerotium rolfsii* always appear in strands or in radial fans.

(8) The individual mycelial threads seem incapable of indefinite growth at the terminal cells of the hypha. New growth is effected by means of a bud developed at the terminal cell of the mycelium.

(9) There is a strong indication of plus and minus strains in *Sclerotium rolfsii*.

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PLATE 3

Sclerotium rolfsii:

A.—Two colonies in same plate, both apparently of the same sexual strain. No sclerotia formed at the point of union of the two colonies.

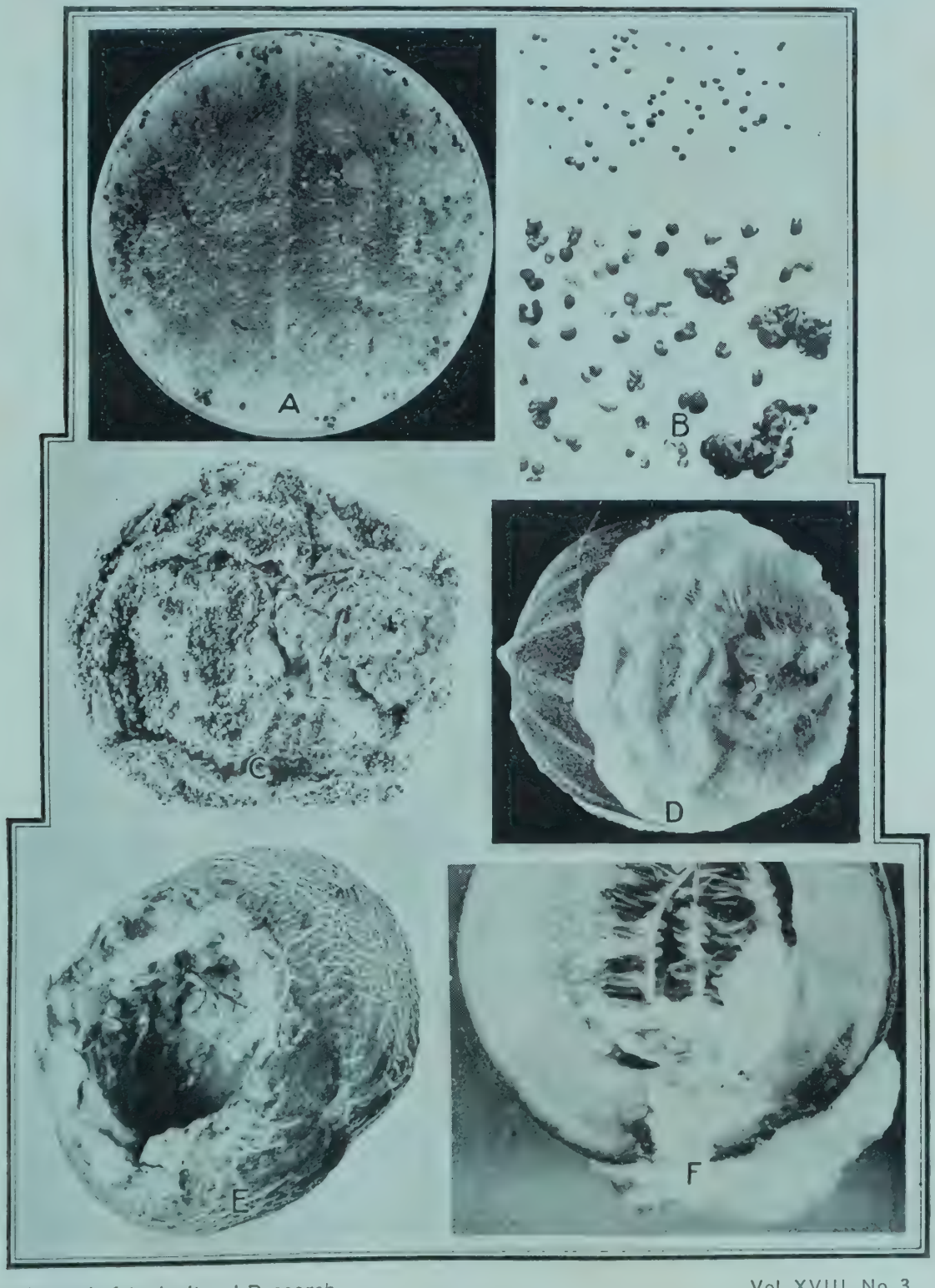
B.—Large sclerotia from inoculated orange.

C.—Late stage, showing cantaloupe reduced to a mass of mycelium and sclerotia.

D.—Inoculated cantaloupe fruit, the point of infection being free and away from the bell jar glass. Earlier stage than C.

E.—Infected cantaloupe, lying close to bell jar at point of infection. This shows the melting away of that part of the fruit in closest contact with the glass, leaving a ragged hole.

F.—Cross section of an infected cantaloupe, showing penetration of fungus into interior of fruit.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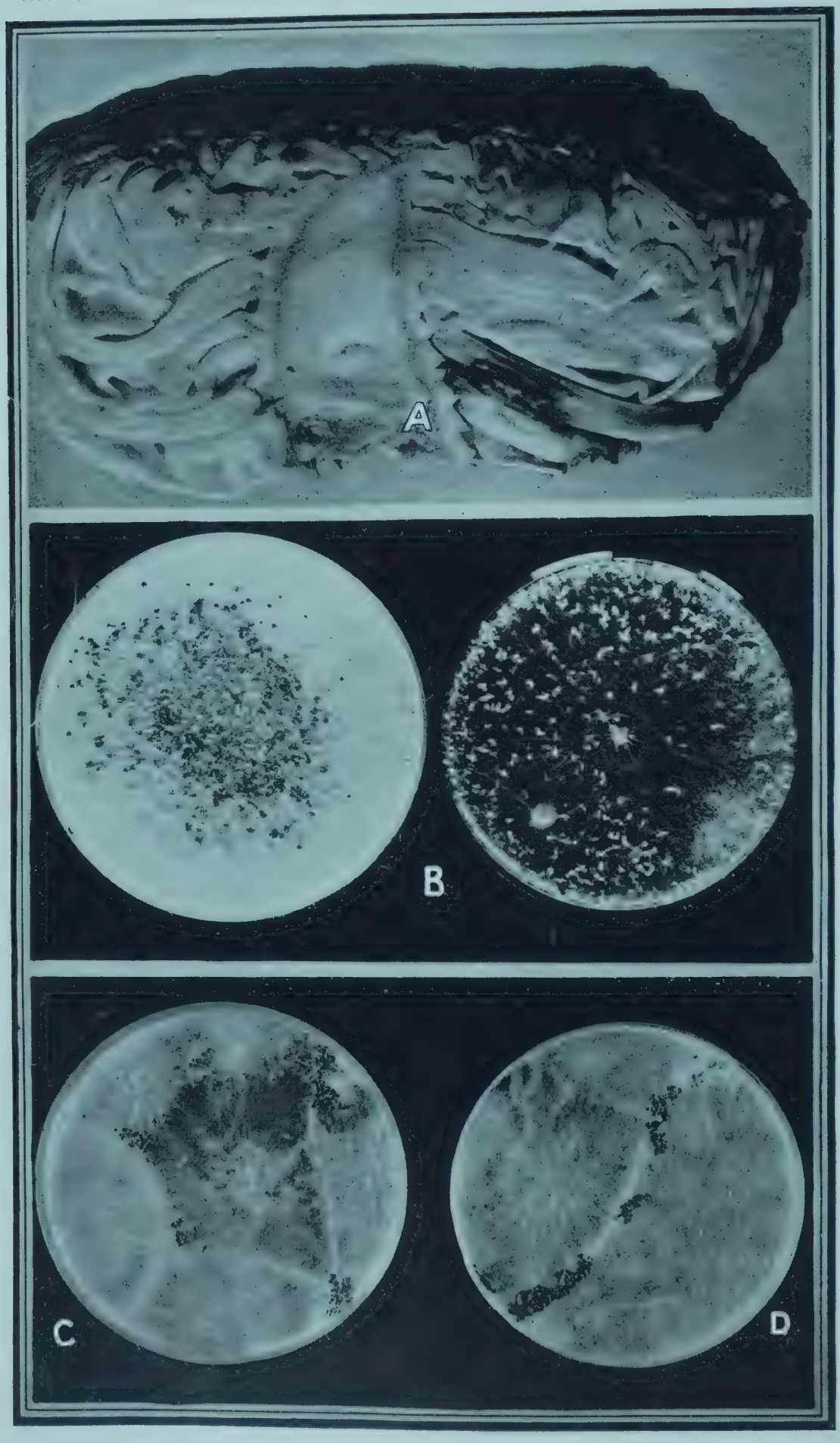
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PLATE 4

Sclerotium rolfsii:

A.—Cabbage artificially inoculated. The rot is confined to the outer layers of the head.

B.—Cultures on artificial media.

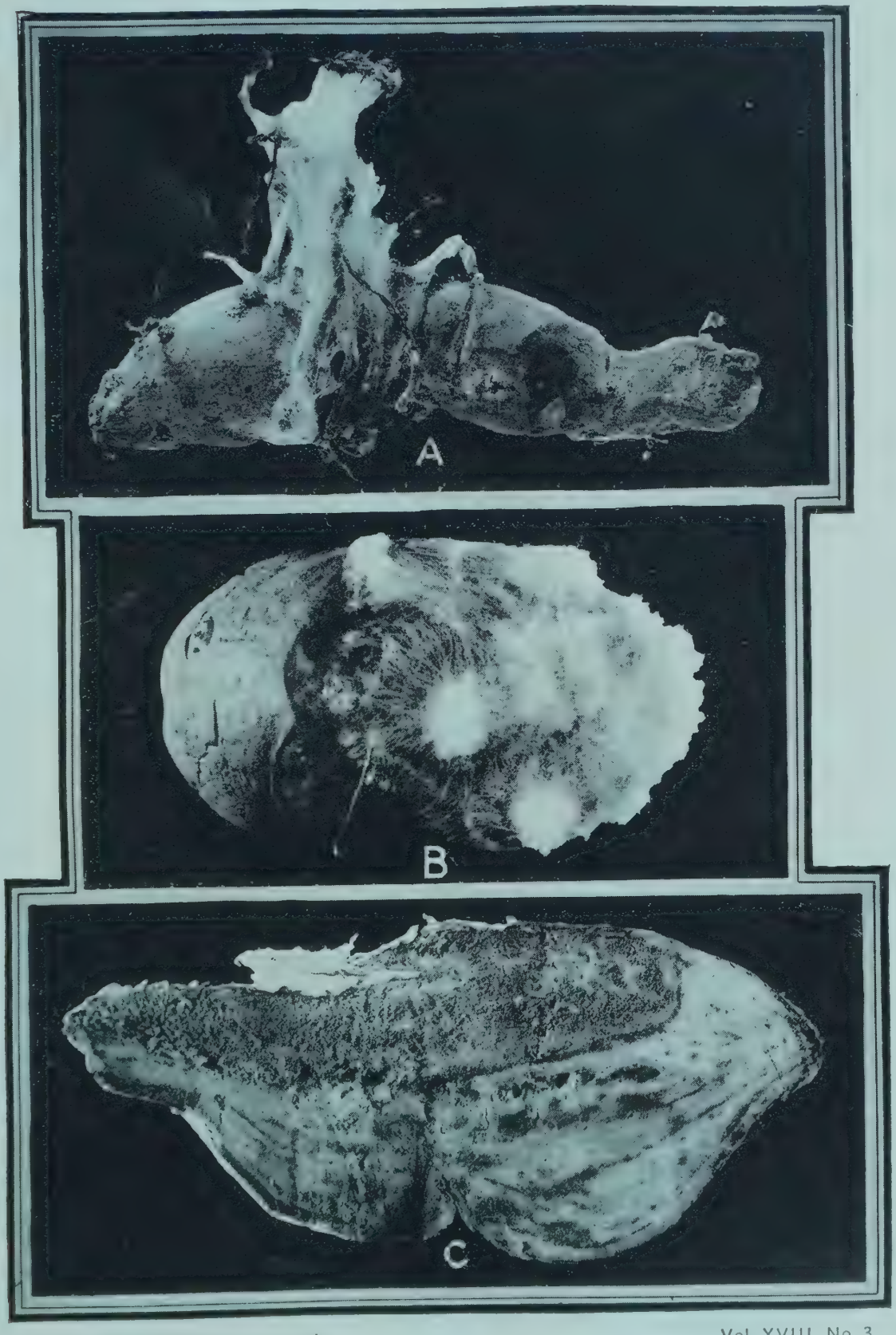
C.—Mustard-seed-like sclerotia on cantaloupe.

D.—Formation of sclerotia at point of union of apparently plus and minus strains.

PLATE 5

Sclerotium rolfsii:

A.—Sweet potato in seed bed, showing method of natural infection of young sprouts.
B.—Infected Irish potato, "melter" stage.
C.—Longitudinal section of infected sweet potato, showing nature of rot.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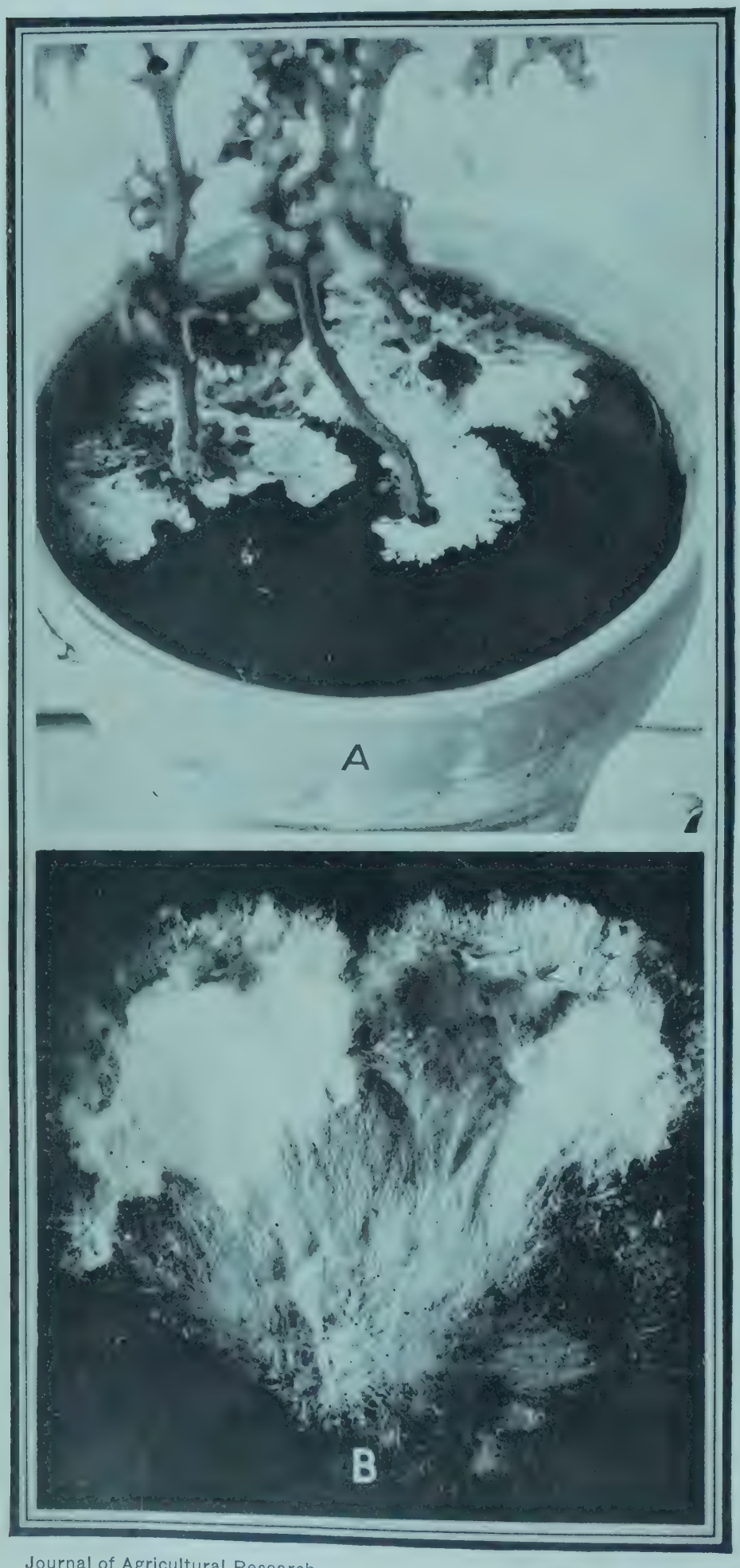
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PLATE 6

Sclerotium rolfsii:

A.—Dead tomato plants, showing fungus growing out on the surface of the soil in radial fans.

B.—Growth on glass of bell jar, showing radial fans.

EFFECT OF VARIATION IN MOISTURE CONTENT ON THE WATER-EXTRACTABLE MATTER OF SOILS

By J. C. MARTIN and A. W. CHRISTIE, *Division of Agricultural Chemistry, California Agricultural Experiment Station*

The question of the possible effect produced on the water-soluble matter by variations in the moisture content of the soil became of interest in connection with investigations of the water extracts of soils carried on at this laboratory.[1] In the work referred to, the soils were maintained at all times as near their optimum moisture contents as was practicable with the large quantities (1,800 pounds) of soil involved. Some variation was, however, found to take place between waterings and is deemed to be inevitable in experiments of that type. The purpose of the present study was to determine to what extent variations in moisture content of soils modify the magnitudes of their water extracts and thus vitiate conclusions drawn from our own and similar experiments.

DESCRIPTION OF SOILS

The two soils studied herein are regarded as typical of the two classes used in much of the recent work of this laboratory. No. 1 is a Yolo silty clay loan and is the same soil as that called "No. 1" in the investigations of Stewart.[1] No. 2 is a sandy loam very similar to the "No. 11" soil described in the same publication. The portions of the soils used in this investigation were taken from bins in which they have been stored for several years and hence show a relatively great accumulation of water-soluble matter. They were practically in the air-dry condition, No. 1 containing 5 per cent moisture and No. 2 about 2.5 per cent. The optimum water content for the silty clay loam soil is 22 per cent, while that of the sandy loam is 15 per cent.

PROCEDURE

Four 500-gm. portions of soil No. 1 were placed in quart Mason jars and brought to a moisture content of 10, 15, 20, and 25 per cent, respectively, making 16 jars or samples in all. The same procedure was followed for soil No. 2, except that the moisture contents were 5, 10, 15, and 20 per cent. These moisture contents were chosen as those covering the range of possible moisture variations of these soils in the field during the season. The lowest moisture content maintained is approximately the air-dry condition, while the highest is slightly above optimum for each soil.

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The jars were then buried in the ground to the level of the soil inside. The covers were set on loosely to allow free circulation of air and at the same time prevent excessive evaporation. The area occupied was shaded to prevent heating the jars and metallic covers by the sun's rays. At frequent intervals—two weeks or oftener, depending on the weather—enough distilled water was added to bring the soil in each jar to its correct moisture content. Since this is a study of the effect of moisture content, it was necessary to keep that factor constant.

Two days after the soils were first moistened, one jar of each soil at each of the various moisture contents was removed and mixed. A 50-gm. portion was dried to constant weight to check the moisture percentage. Soil equivalent to 340 gm. in the water-free state was extracted with 1,700 cc. of distilled water, inclusive of the water in the soil, thus insuring a 1 to 5 extraction, which was made after the manner described by Stewart.[1] The remainder of the soil was used to determine the concentration of the soil solution, by the freezing-point lowering, as described by Bouyoucos and McCool.[2] This procedure was repeated three times during the period of the experiment at varying intervals of time; the total length of time between the first and last sets of analyses was 22 weeks, which is longer than a normal growing season.

Table I gives a complete résumé of the results obtained. The concentration of the soil solution is reported in atmospheres of osmotic pressure, the analyses of the water extracts as parts per million of the water-free soil.

DISCUSSION OF RESULTS

Knowledge of the inherent variations in the methods used is very essential in a study of this nature. For a detailed description of the methods employed reference is made to two recent publications from this laboratory.[3] It is desirable to draw conclusions as to differences only where such variations are of considerable magnitude, 20 per cent or more. It is also evident that comparisons should be made only between results obtained at the same sampling date, since it is known that there are seasonal fluctuations due to other factors.

OSMOTIC PRESSURE OF THE SOIL SOLUTION AND TOTAL SOLIDS.—These two determinations are placed together for consideration because of the fact that they are known to be directly related, as has been shown by Hoagland.[4] The most striking feature shown is the decrease of soluble matter in soil

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[4] HOAGLAND, D. R. THE FREEZING-POINT METHOD AS AN INDEX OF VARIATIONS IN THE SOIL SOLUTION DUE TO SEASON AND CROP GROWTH. *In* Jour. Agr. Research, v. 12, no. 6, p. 369–395, 8 fig. 1918. Literature cited, p. 394–395.

No. 2, 20 per cent moisture. It might be stated here that this moisture content produced a water-logged condition, which will be discussed later. The percentage of decrease is noticeably greater at the later sampling dates than at the earlier, 20 per cent on May 13 and 60 per cent on October 28; and there is reason to believe they would continue to increase in divergence. Although that is the most striking feature, it is also noted that there is a general trend upward from the lowest moisture content to the optimum in both soils.

TABLE I.—*Concentration of soil solution*

SOIL NO. 1, SILTY CLAY LOAM

Moisture content.	Date of sampling.	Osmotic pressure.	Total solids.	Nitrate (NO_3).	Sulphate (SO_4).	Phosphate (PO_4).	Potassium (K).	Calcium (Ca).	Magnesium (Mg).
Per cent.		*Atmos.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
10	May 13	1.67	1,208	244	194	3.8	54	90	51
	June 11	1.57	1,163	239	190	4.2	32	101	43
	July 22	1.35	1,422	332	186	2.9	36	99	44
	Oct. 28	1.35	1,341	274	201	3.7	41	112	69
15	May 13	1.50	1,262	240	212	3.3	58	90	51
	June 11	1.42	1,287	287	211	3.0	38	102	41
	July 22	1.95	1,547	283	213	2.8	40	105	54
	Oct. 28	1.79	1,476	291	219	4.0	57	113	85
20	May 13	1.55	1,362	244	225	3.7	62	92	47
	June 11	1.66	1,317	309	223	3.2	43	97	48
	July 22	1.66	1,553	327	221	3.2	40	100	53
	Oct. 28	1.72	1,482	289	244	2.6	52	122	61
25	May 13	1.32	1,300	220	228	3.5	57	90	45
	June 11	1.70	1,345	245	226	3.0	46	102	47
	July 22	1.88	1,566	237	217	2.3	43	99	45
	Oct. 28	2.11	1,502	252	240	4.1	54	121	65

SOIL NO. 2, SANDY LOAM

5	May 13	0.42	494	132	52	4.7	37	47	17
	June 11	.74	541	124	46	4.6	28	67	13
	July 22	.59	715	131	50	4.5	29	62	17
	Oct. 28	1.01	613	159	52	7.4	38	80	16
10	May 13	.56	504	128	54	4.0	43	50	17
	June 11	.79	561	133	49	3.2	31	63	13
	July 22	.55	718	162	54	4.1	35	70	19
	Oct. 28	1.04	685	190	56	9.5	45	85	15
15	May 13	.54	541	135	54	4.3	40	50	18
	June 11	.85	579	133	49	3.7	32	66	13
	July 22	.72	786	176	51	4.6	37	71	19
	Oct. 28	1.02	773	210	60	5.0	43	90	18
20	May 13	.21	415	64	53	6.1	42	42	15
	June 11	.09	321	6	50	3.5	26	37	7.5
	July 22	.06	375	Trace.	48	5.3	28	40	16
	Oct. 28	.06	307	4	15	5.6	34	59	12

NITRATES.—The most notable thing is the depression in soil No. 2, 20 per cent moisture. This solute is the most affected by the excess water, as might be expected, anaerobic conditions having been produced. Another feature is the fact that at the optimum moisture content there is found the greatest quantity of nitrates, especially emphasized in soil No. 2 at the two later sampling dates.

SULPHATES AND PHOSPHATES.—The excess water does not have a depressing effect on these two solutes. The only evidence of any difference in quantity is the general trend upward in the water-soluble sulphate, coincident with the increased moisture in soil No. 1, which is relatively high in sulphate.

POTASSIUM.—There is a trend upward in quantity of this solute in soil No. 1 from the lowest to the highest moisture content; in soil No. 2 no significant effect is produced in the first two sets of determinations; however in the two latter it is seen that there are increasing quantities of potassium from soils of 5 per cent to 15 per cent moisture, and a decrease in the soil of 20 per cent moisture.

CALCIUM AND MAGNESIUM.—The striking feature is the depressing action of the excess water in soil No. 2 and the almost identical percentages of depression for the two solutes at the corresponding sampling date.

GENERAL DISCUSSION

In preparing the water extracts it was observed that the extract of the soil at the lowest moisture content filtered much faster than that at the highest, with a regular gradation between the two extremes, and that the difference in filtering speed was greater between the silty clay loams of highest and lowest moisture content than between corresponding samples of the sandy loam. A reasonable explanation may be found in the physical structure of the soil at the different moisture contents. It has been pointed out by Fippin[1] that the continued wet condition or dry condition does not produce any change in structure, but that alternate wetting and drying produce granulation, caused by expansion and contraction, which are directly proportional to the degree of wetting or drying. Thus the dryer silty clay loam may approach in structure that of a sandy loam. Klein[2] suggests that the increased granulation and hence greater access of water to the soil particle by drying may be overcome by the greater amounts of material held soluble in the soil at the higher moisture contents.

When a condition of saturation is reached there is a marked depression, as has been noted. The sandy loam soil at 20 per cent moisture was saturated, and water was standing on its surface a quarter of an inch in depth. This in itself is an indication of the results which might have been expected, and which were in fact obtained. This soil contained 5 per cent more water than its optimum, while soil No. 1 with 25 per cent moisture was 3 per cent above its optimum content and showed no apparent depression of solutes. Silty clay loam soils have a greater range between their optimum moisture contents and their saturation points than sandy loam soils because of the difference in soil texture.

The range of variation in moisture content covered in the present study was very much greater than the variations occurring in the investigations[3] referred to in the early part of this paper. In a season's work with these two soils it was observed that for soil No. 1 the moisture con-

[1] FIPPIN, Elmer O. SOME CAUSES OF SOIL GRANULATION. *In* Proc. Amer. Soc. Agron., v. 2, 1910, p. 106–121, fig. 11–18. 1911.

[2] KLEIN, Millard A. STUDIES IN THE DRYING OF SOILS. *In* Jour. Amer. Soc. Agron., v. 7, no. 2, p. 49–77, 3 fig. Bibliography, p. 75–77. 1915.

[3] STEWART, Guy R. OP. CIT.

tent variation was between 22 per cent and 16 per cent, with an average of 19 per cent; for soil No. 2, the extremes of moisture variation were 16 per cent and 11 per cent, with an average of 13 per cent. These are variations of approximately 5 per cent from the optimum moisture contents and show the tendency of the soil to be slightly below the optimum in moisture. In the present study the only significant variations from the maximum quantity of extractable material are observed when the soils are either approaching the air-dry condition or are in a moisture-saturated condition. Looking again at the results recorded in the table and calling especial attention to those of soil No. 1 at 20 per cent moisture, at 15 per cent, and even at 25 per cent, and also to those of soil No. 2 at 15 per cent and at 10 per cent—these being variations of from 5 to 7 per cent below their optimum moisture contents—it is readily seen that at any one sampling date the quantities of water-extractable materials are exceedingly uniform and represent no significant differences. Therefore it is concluded that in water-extraction studies of soils the moisture content at which the soils are maintained need not necessarily be limited to a narrow range.

SUMMARY

(1) The water-soluble constituents of two soils of very different type have been studied at four moisture contents.

(2) The moisture contents approaching the air-dry condition show a decided tendency to depress the nitrates and potassium in both soils and the sulphates in the silty clay loam only. These depressions are reflected in the total dissolved material.

(3) The excess water in the sandy loam soil causes a disappearance of nitrates and also decidedly depresses the potassium, calcium, and magnesium, these losses also being reflected in the total solids extracted.

(4) Considerable variations in moisture contents of soils, provided the saturation point is not reached, do not appreciably modify the results obtained by the water-extraction method.

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PATHOLOGY OF DOURINE WITH SPECIAL REFERENCE TO THE MICROSCOPIC CHANGES IN NERVE TISSUES AND OTHER STRUCTURES

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Within the last five years on an average of from 45,000 to 50,000 complement-fixation tests have been made annually by the Bureau of Animal Industry for the diagnosis of dourine. The samples have been forwarded from Montana, North Dakota, South Dakota, Nebraska, Wyoming, Arizona, and New Mexico, especially from the Indian reservations in those States. Many improvements have been made in perfecting the technic of the complement-fixation test, thus affording better means of diagnosing dourine, which is the most essential factor in eradicating the disease. Diagnosis is the chief aim; but while all energies are directed toward that end other phases of dourine should not be overlooked, since they may help to explain directly or indirectly the variations in existing symptoms and the changes produced in the course of the disease. A careful perusal of the literature on this disease reveals the fact that in most articles on dourine the clinical picture—etiology, symptoms, and treatment—receives more attention than the pathological phase, the microscopic changes. These changes are often disposed of in a few sentences or short paragraphs, quoted usually from the older European descriptions of the microscopic findings. Very little consideration and study has been given in this country to determine whether microscopic changes as described in European cases of dourine are identical or different.

The object of this paper is to describe the microscopic lesions found in nerve tissues and other affected parts. The materials studied was taken from several well-developed chronic cases of dourine in horses from Montana and Iowa in which the disease had been recognized clinically and the animals had reacted to the complement-fixation test. The animals were shipped to the Bethesda, Md., experiment station of this bureau and kept under observation for nearly two years until they died. On post-mortem examination these animals showed lesions of dourine. An attempt will be made in this preliminary paper to correlate the clinical symptoms with the microscopic findings. In fact, the clinical symptoms afford chief guidance in selecting the tissues in which the structural changes were studied.

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One of the difficulties confronting workers with nerve tissues is that in a short time post-mortem changes set in, and the beginning dissolution of nerve tissues may appear. Only the experienced technician accustomed to handling nerve tissues can apprehend the consequences. It is for this reason that the details of neurological technic will be described to enable the reader to follow the various steps necessary in bringing out all the details of the finer cytological changes which may occur in the tissue as the result of disease.

IMPORTANCE OF PROPER FIXATION

Before proceeding to make a post-mortem examination, proper fixing solutions must be ready to avoid all danger of disintegration. Fixation is a process by which the tissue is quickly killed and its structure rendered permanent.

The fixing agent must have the power to penetrate quickly before post-mortem changes have begun, as well as to give permanent results without distorting the shape, size, and position of the tissue elements. A fixing fluid of alkaline reaction should be avoided, since it tends to dissolve certain structural constituents rather than to fix. Some reagents, such as alcohol, while fixing rapidly cause a violent shrinkage of the tissue by inducing an unbalanced exosmosis of the fluid cell contents. In this way they bring about the shrinkage or collapse of the cells, which is decidedly objectionable.

The aim therefore should be to select fixatives in which the ingredients are mixed in such proportions that the swelling tendency of one will counteract the shrinkage tendency of the other. In removing nerve tissue, care must be taken that the portions exposed do not become dry and that the parts removed are not crushed or stretched. No single fixing fluid and no single staining process suffices to bring out finer structural changes. The neuroplasm of the ganglion cells is different from the neuroplasm of the nerve fibers not only in its appearance, composition, and behavior toward different fixatives but also in its affinity for different stains. Chromatophil granules are present in the ganglion cells but not in the nerve fibers. Myelin is present in the nerve fibers, but not in the nerve cells. Held has pointed out that chromatophil granules are brought out by the treatment of nerve tissue with alcohol or certain other fixing fluids. These appear according to their treatment as fine or coarse objects but are not visible in fresh nerve cells. The finer cytologic changes which are at the bottom of nervous diseases can be demonstrated only by modern methods of staining.

The structural distinction of nerve cells and nerve fibers is made as a matter of convenience for classification, description, and anatomical correlation. In reality the nerve cell and the nerve fibers constitute collectively the unit of the nervous system. This unit, called the neuron, is

composed of a cell body, nucleolus, nucleus, chromatic substance, achromatic substance, pigment, and processes. It would also include the protoplasmic processes with their gemmules and the axis-cylinder process, its collaterals, and at times a final arborization. The term chromatic or chromatophil substance, or Nissel's bodies, is applied to that portion of the cell substance which stains and has an affinity for methylene blue. It presents itself in many forms as irregular particles, smooth or dented fibers, or dumb-bell-shaped masses. The function of the chromatic substance has not up to the present time been definitely determined. The achromatic substance constitutes the greater part of the cell body and is made up of fine fibrils which pass through the cell with numerous anastomoses which gives this substance a finely reticular appearance. Certain observers consider the fibrils as the continuations of axones on their way into the processes. According to this view the achromatic substance is the all-important part of the cell.

The nerve fibers, on the other hand, are not independent elements but are those processes of the neuron known as the axon or axis-cylinder processes, which after their exit from the cell body extend and become invested in a protecting sheath. They are then known as the medullated nerves. The essential part of the nerve fiber is the central cord or axis cylinder, which is the only part concerned in transmitting the nerve impulse. The axis cylinder is composed of most delicate axis fibrillae embedded within a semifluid interfibrillar substance and is surrounded by a delicate sheath, the axilemma. The axis cylinder is surrounded on the periphery by a relatively thicker coat, the medullary sheath or white substance of Schwann, outside of which lies the delicate enveloping coat, the neurilemma. The medullary sheath is composed of a delicate reticulum of neurokeratin, the meshes of which are filled with a fatty substance, the myelin. This constitutes the majority of the peripheral medullated nerve fibers, but the medullated fibers of the spinal cord have no neurilemma.

The nerve fibers in the sympathetic system have no medullary sheath and are spoken of as the nonmedullated or gray fibers. The nerve cells and nerve fibers within the central nervous system are held together by a special supporting tissue, the neuroglia, which consists of an interlacing network of extremely fine fibrillae, the glia fibers, and glia cells. The cells are irregularly scattered in the course of the glia fibers.

Of the different fixing fluids, a 4 per cent solution of formaldehyde was used as a preliminary fixative more often than any other on account of its great power of penetration and rapid fixation. It must be followed, however, by other reagents such as Müller's fluid or Zenker's fluid, containing chrome salts, which bring out more clearly the ganglion cells, neuroglia, and axis cylinder. The chrome salt probably enters into chemical combination with the myelin, thus making possible the use of differential myelin sheaths stain.

Besides the general nuclear stains for cell protoplasm, selective stains were used, as the Van Gieson's stain, Nissel's stain, Pal's modification of Weigert's myelin stain, and Marchi's method of staining fatty degeneration in the myelin sheaths.

MANIFESTATIONS OF DOURINE

In reviewing the etiology of dourine we find that the unicellular protozoan *Trypanosoma equiperdum* is considered the cause of dourine. It is apparent that the disease, being transmitted in the act of coition, should show the principal lesions in the genital organs. But in all chronic cases of dourine, besides the lesions in the sexual organs pronounced derangements of peripheral nerves and the central nervous system are present, manifested by paralysis of nerves and atrophy of various groups of muscles. As trypanosomes can be found neither in the central nervous system nor in the peripheral nerves it must be assumed that the trypanosomes elaborate poisonous products or toxins which are responsible for the lesions. The presence or absence of lesions in acute cases will not be discussed, since all the observations were confined to chronic cases, nor will the symptoms and post-mortem examination be described in this paper.

The following tissues were selected: Brain, spinal cord, spinal ganglia, and peripheral nerves. No detailed study of muscles, skin, and genital organs was undertaken, though some preparations of these structures were made.

BRAIN

A number of pieces were taken from various parts of the brain, principally in the region of the lateral fissure (Sylvius), supersylvian fissure, perisilvan fissure, and the sulcus rhinalis. Some of the sections were stained by the Van Gieson method, others with the Erythrosin-tuolin blue method. Both methods are used to study the general morphology of nerve structures, particularly the cell bodies of neurones. The nuclei appear bluish red, the ganglion cells and their protoplasmic processes red, the axis cylinder brownish red, the myelin sheaths yellow, the neuroglia fibers orange red, and the connective fibers deep red.

The sections from the different parts did not reveal any appreciable abnormality either in the nerve cells and their pericellular lymph spaces or in the blood vessels and their perivascular spaces. The cell and the nucleus took the stain uniformly; their size and external contour were unaltered. The blood vessels also appeared normal. Staining with the Nissel method showed no change in the chromatophil granules; neither was their amount or size increased or diminished. There was no change in regard to the staining ability or grouping of the nucleus or any indication of chromatolysis. Staining with Pal's modification of the Weigert method showed good contrast of the gray and white substance but no

evidence of degeneration. Staining with the Marchi method showed very light-brownish or yellowish coloration of the myelin and no black deposits. This differential action of osmic acid results from the fact that chromic acid and its salts deprive the normal myelin sheaths of their power of reducing osmic acid, while the chemically changed degenerating or abnormal sheaths retain this power. The method therefore gave positive black images of fibers in a state of degeneration, while the normal fibers remained unstained or only with light-colored yellow due to the bichromate in the Müller's fluid.

SPINAL CORD

The spinal cord contributed the bulk of material for the study of nerve changes. A number of pieces were taken from the cervical, thoracic, lumbar, and sacral regions. These pieces were fixed by methods best adapted for each particular stain. Fewer pieces were taken from the dorsal region than from the cervical or lumbar, as was indicated by the clinical symptoms and borne out by the microscopic examination. The sections from the anterior and middle dorsal region when stained with the Van Gieson method showed good contrast between gray and white substances, unaltered motor-ganglian cells, and no increase or reduction in neuroglia tissue or in the size of the medullated nerve fibers which constitute the dorsal, lateral, and ventral columns. The use of Nissel's method showed the chromatophil granules well stained, unaltered in amount, and with no indication of any degenerative changes. Pal's modification of the Weigert and the Marchi methods failed to show any evidence of alteration in the myelin in the medullated nerve fibers. The blood vessels appeared normal. Sections from the cervical and posterior dorsal regions will be discussed at the same time, since they showed similar lesions.

In sections from these regions stained with the Van Gieson method no appreciable changes could be observed either in the multipolar nerve cells or in the medullated nerve fibers of the gray or white substance. The Nissel method showed the chromatophil granules fairly well stained, slightly disarranged, but not enough to produce a significant deviation. Pal's modification of the Weigert method did not show sufficient difference in the medullated fibers to attach much importance to the lack of contrast between the gray and white substances. In sections stained by the Marchi method some of the medullated fibers in the dorsal horns showed a few scattered black clumps, especially in the extramedullary fibers outside the gray substance near the dorso-lateral groove. This was the first indication of degeneration of the myelin. It could not be detected by the other methods of staining. The medullated fibers of the ventral horns showed no black clumps. There were no black clumps in the medullated fibers of the dorsal columns in the white substance

In sections from the lower lumbar and sacral region stained by Van Gieson's method the deviations were more apparent than in the lumbar region.

The dorsal and ventral horns and the gray commissure appeared to have an increased quantity of neuroglia fibers. The Rolandic substance capping the dorsal horns was more distinct. The central canal in the middle of the gray commissure was enlarged. The single row of ependyma cells lining the canal were somewhat flattened, probably by pressure of spinal fluid, which distended the central canal. The central gelatinous substance, which is a modified neuroglia surrounding the central canal, appeared to be increased in amount and to contain hypertrophied glia cells. Simple dilatation of the central canal of the spinal cord is known as hydromyelia. When the dilatation becomes very extensive it is difficult to distinguish this condition from the hollowing out of the central canal by a process of softening known as syringomyalia, which is however usually found in the cervical region. Neither the motor-nerve cells of the ventral horns, the sensory-nerve cells within the dorsal horns, nor the cells of the column of Clark showed marked abnormality when stained by the Van Gieson method. There was, however, an increase of neuroglia tissue in the vicinity of the dorsal median groove, dorso-lateral grooves, and the entrance and course of the dorsal nerve roots. This increase of neuroglia was not sufficient to constitute sclerosis of the dorsal column. Sections that were stained by the Nissel method showed what may be regarded as the beginning of chromatolysis. The constituents that are most susceptible to influences are the Nissel bodies or chromatophil granules. While the mechanism of chromatolysis is still obscure, it is generally believed that the process represents the reaction of the cells to the disturbing forces, resulting in disintegration of the chromatophil granules in various parts of the cell, and is spoken of as peripheral, perinuclear, and disseminated.

Slight peripheral and perinuclear disintegration was observed in the sensory-nerve cells and to a less degree in the motor-nerve cells and the cells from the column of Clark. Neurologists generally consider that this latter condition is reparable or that the functional activity of the nerve cells is only partly impaired; but when the cell becomes deprived of its functional activity a further step in chromatolysis is produced that is not reparable, constituting a later stage of degeneration known as acromatolysis or plasmolysis. This latter condition was observed in sections from the lumbar region. No other method was so sensitive as the Nissel method in showing the earliest change of chromatolysis in the ganglian cells. Sections stained with Pal's modification of Weigart's method showed slight alterations. The bluish slate color of the medullated nerve fibers of the white substance showed good contrast with the yellowish stained gray substance which has only a limited number of medullated

fibers. The contrast was better seen in the ventral and lateral columns and was less apparent in the dorsal column where the medullated fibers appeared to be of a faded slate color approaching a yellowish tint, thus bordering on a beginning stage of degeneration. The change was sufficient to indicate a degeneration.

The Marchi method and Robertson's modification of Heller's method, both containing osmic acid, showed decided degeneration of the myelin in the medullary sheath of the nerve fibers. The intermedullary fibers within the gray substance contained a fair number of black clumps in the dorsal portion of their course. The extramedullary sensory fibers constituting the dorsal root showed more black clumps at the point of their entrance, the dorso-lateral groove. The black clumps gradually became fewer as the fibers entered the dorsal horns. The intramedullary fibers of the ventral horns, as well as their extramedullary portion, the motor-nerve roots, had only occasionally some black clumps. The distribution of these clumps in the medullated fibers of the white substance deserves special attention on account of the columns that are involved and the deduction of symptoms that could be made from a clinical standpoint.

The principal manifestations were found in the outer portion of the dorsal column, which is known as "funiculus cuneatus" or "Burdach's column." The black clumps were more numerous in the medullated fibers forming the outer boundary of the column or the fibers close to the dorsal-nerve roots and the dorsal horns. Fewer black clumps were present in the fibers near the inner boundary. The inner portion of the dorsal column is known as "funiculus Gracilis" or "Goll's column." Fewer black clumps were present than in the outer Burdach's column. Their number diminished as the fibers approached the dorsal medium septum. The medullated fibers of lateral and ventral columns showed no black clumps, except in the vicinity of the tips of the ventral horns. The changes observed in sections from the lower lumbar region were similar to the changes present in the sacral region. The degree of degeneration was a little more marked in the sacral region than in the lower lumbar region. The staining with the Marchi method and Robertson's modification of Heller's method showed the degeneration of the myelin in the medullary sheaths more pronounced and especially in the column of Burdach and to a less degree in the column of Goll, where the black clumps decreased in number. The black clumps gradually became fewer and disappeared entirely toward the distal end of the sacral region.

In man the subdivision of the white matter into various tracts with defined conducting pathways by which nerve impulse is conveyed has been worked out by research based on combined evidence of anatomical, pathological, and embryological investigation; but our knowledge of

these tracts in domestic animals is quite limited. We must recognize, however, in the white matter three classes of nerve fibers: those entering the cord from the periphery of the body, those entering from the brain, and those arising from the nerve cells situated within the cord itself. Fibers constituting the pathways for the transmission of impulses from lower to higher levels form the ascending tracts, while those fibers in which the impulse is conducted in the opposite direction form the descending tracts. The medullated fibers in the column of Burdach and the column of Goll were more affected than the fibers in other columns. The fibers of the dorsal column consist of two sets of axones. The afferent or sensory axones, which come from the cells of the spinal ganglia, enter as the dorsal roots of the spinal nerves and divide into two branches. The anterior ascending branches from the sensory pathway to the brain, extend to the fasciculus cunlatus and fasciculus gracilis, or corresponding tracts, to the nuclei in the medulla oblongata. The posterior descending branches extend backward for varying distances and give off numerous collaterals to the cells of the gray column, thus forming part of the mechanism for the mediation of reflex action. Some collaterals cross in the white commissure to the opposite side. The second set of axones arise from the smaller cells of the gray column. They enter the white matter and divide into anterior and posterior branches, forming the fasciculi proprii or ground bundles of the cord. The function of this set of axones is chiefly to associate various levels of the cord.

SPINAL GANGLIA

In removing the spinal cord, the spinal ganglia of the cervical and dorsal regions became detached. The ganglia of the lumbar enlargement and the sacral region alone were saved so that only a limited number of these ganglia could be examined. The same stains were used as for the staining of the brain and spinal cord. All ganglia appeared to be enlarged. The capsules were thickened. Many of the peripherally disposed nerve cells appeared normal in size. The nuclei and the chromatophil granules were well stained in some and much paler in others where the chromatophil granules were not only reduced in size but almost disintegrated and scattered among these average-sized cells. Smaller, irregular, shrunken cells were present, in which the disintegration was more marked. These latter cells took the staining very poorly and had the nucleus displaced toward the periphery. But even in these cells the nuclei had not entirely lost the staining property. The partial disintegration of the chromatophil granules and the pale color of the shrunken or sclerotic cells indicated varying degrees of chromatolysis. The interstitial tissues in the interior of the ganglia were increased in amount and showed in places clusters or groups of round-cell accumulations. In the sacral ganglia a greater number of shrunken cells were

present and chromatolysis had reached a stage where the chromatophil granules had disintegrated and in some of the cells totally disappeared. Here the name of plasmolysis is more applicable.

EXTRASPINAL NERVE TRUNKS

The great sciatic nerve was the only nerve trunk examined. The nerves from both sides were taken in each case, and transverse and longitudinal sections were made. The same stains were used as for the brain and cord, except that the Nissel method was omitted. The transverse sections were more instructive than the longitudinal. A number of the medullated fibers showed degeneration. A cross section of the fiber appeared as a circle with a dot in the center, corresponding to the axis cylinder. In many of the fibers the degeneration was so complete that the myelin as well as the axis cylinder disintegrated completely, leaving a granular mass behind. The circular outline of the cut fiber could not be distinguished. The funiculus therefore contained fewer circles which were separated by disintegrated material. The endoneurium was slightly increased in amount. The perineurium was more increased and the outlines of the individual funiculi became more distinct. There was also an increase in the number of connective tissue cells. The epineurium was more hypertrophied than the perineurium or the endoneurium. Scattered between the funiculi a number of perivascular inflammatory foci were present, besides the increase of irregularly scattered connective tissue cells. The left sciatic showed a greater number of degenerated fibers than the right. The Marchi method showed a fair number of black clumps in the interior of the funiculi. In longitudinal sections the black clumps were arranged in continuous rows in the more peripherally disposed funiculi. In the left nerve the rows of black clumps were longer, and more fibers were affected than in the right nerve.

SUMMARY

The microscopic examination of the brain showed no appreciable changes in the nerve cells, the supporting tissue, or in the blood vessels. In the cervical, anterior, and middle dorsal portions of the spinal cord lesions could not be demonstrated even with the most sensitive methods of staining; and in the posterior dorsal portion the lesions were very slight, gradually increasing in the lumbar enlargement and becoming most marked in the sacral region. Degeneration in the sensory ganglion cells was present in all stages, varying from the beginning stage of chromatolysis that could barely be detected by the Nissel method alone to advanced degeneration and disintegration of plasmolysis that was brought out by less sensitive methods. The motor ganglion cells and the cells in the column of Clark showed such slight alteration that it was difficult to trace chromatolysis in them. The nerve cells of the spinal

ganglia showed chromatolysis in varying degrees. The most marked changes were found in the sensory cells in the sacral region where disintegration of the chromatophil granules was followed by atrophy and sclerosis and was invariably accompanied by peripheral displacement of the nuclei. This was not observed in the nerve cells of the cord.

The degeneration of the myelin in the medullated fibers was even more pronounced than the degeneration in nerve cells. The black clumps of degenerated myelin stained by the osmic acid of the Marchi method were the characteristic feature of the endoneural and extraneural fibers in the gray substance in the dorsal horns and the dorsal nerve roots as well as of the fibers of the columns of Burdach and Goll in the white substance of the cord. The changes were limited to the lumbar and sacral region. In the sciatic nerve the degeneration was even more marked. We can therefore assume that the disturbances are of peripheral rather than central origin.

YELLOW-BERRY IN HARD WINTER WHEAT

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In an earlier publication[1] of the Kansas Agricultural Experiment Station, data were presented to show that yellow-berry in wheat is heritable and that improvement in the ability of wheat to resist the disease can be accomplished by breeding. The investigations reported therein have been extended, and some studies pertaining to the physiological processes that result in yellow-berry have been made. It is the purpose of this paper to present the results of these later studies.[2]

THE NATURE OF YELLOW-BERRY

The yellow-berry problem has two aspects, the one practical, the other theoretical. The presence of yellow-berry in wheat causes it to grade lower and sell at a lower price than clear, hard wheat high in gluten. Furthermore, as Bailey says (*1, p. 18*):

> If the kernels are soft in texture, or represent what is termed the "yellow-berry" condition, the percentage of flour will be reduced, since it is mechanically impossible to free the bran from the floury portions so nearly as when the endosperm is hard and vitreous.

It is apparent that if the yellow-berry condition were eliminated from hard wheats, the practical interests of both the grower and the miller would be subserved.

The term yellow-berry has been defined by Roberts and Freeman (*6, p. 1*) as—

> the appearance [in hard, flinty wheats] of grains of a light yellow color, opaque, soft, and starchy. These opaque yellow grains, constituting what are called the "yellow berries," may have this character throughout; but sometime from a small fraction to half of a grain will be yellow and starchy, while the remainder of the kernel will be hard, flinty, and translucent. The difference in color between the flinty grains and the "yellow berries" is due to differences in the structure and contents of the cells of the endosperm.

The cause of yellow-berry in wheat has been the subject of some investigation. It appears to have been reported first by Bolley (*2, p. 35–36*), who held that the opaque, yellow spotting of the kernels was due not to heredity but to climatic factors, and that—

> this peculiar mottling is due to the action of moisture, air, and sun upon the grain while it is yet in the chaff. If the weather action is long continued, the grains become evenly bleached over the entire surface. The color and hardness of the grain can be maintained by proper care in harvesting and curing.

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[1] Reference is made by number (italic) to "Literature cited," p. 169.

[2] Credit is due the Department of Chemistry for the chemical analyses reported herein.

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Lyon and Keyser (*5, p. 25–29*) came to the conclusion that—

there is quite a definite relation between the per cent of yellow berries in the crop and the character of the season in so far as the latter affects the date of ripening, the composition, and the yield of wheat.

From experimental data they find that—

the amount of "yellow-berry" increases with the lateness of ripening,

and that—

crops of large yield and low nitrogen content contain more "yellow-berries" than do crops of the opposite kind.

They conclude that—

since it has been shown that the amount of yellow-berry increases as the ripeness of the grain increases, and also with the length of time the cut grain is exposed to the weather, it is impossible to lessen the loss by cutting the grain rather early and stacking as soon as sufficiently dry.

Roberts and Freeman (*6, p. 21–35*) found that in two successive years there was a diminution in the amount of yellow-berry corresponding to the shortening of the fall growing period on account of late planting. No relation was found to exist between the spring growing period and the percentage of yellow-berry, except that, in general, late ripening increased it. Higher mean temperatures for the three weeks before ripening were found to be correlated with low percentage of yellow-berry. Evidences of hereditary tendencies were found.

Headden (*4, p. 30–37*) studied the effects of different commercial fertilizers on yellow-berry. His results may be summarized in his own words, as follows:

In our case it is evidently the ratio between the potassium and nitrogen which determines the presence of yellow-berry. . . . The degree of mealiness or starchiness, the yellow-berry, . . . depends upon the relative available supply of these two elements. . . . The application of nitrogen, which was in the form of sodic nitrate, greatly reduced the amount of yellow-berry, in some cases preventing it altogether.

Headden does not find that climatic conditions, the soil, or the amount of available phosphorus affects the development of yellow-berry, but states that—

it can be greatly intensified or increased by the application of available potassium,

and that—

yellow-berry indicates that potassium is present in excess of what is necessary to form such a ratio to the available nitrogen present as to be advantageous to the formation of a hard, flinty kernel. . . . I do not think that there can be any question of the identity of this affection of our wheat with that of Kansas, Nebraska, or South Dakota, and almost no question but that the opaque wheats of California and the Pacific Coast States in general are identical in their character with extreme cases of yellow-berry in Colorado and have the same cause.

This last phase of the question has not yet received much attention. The fact that yellow-berry is produced under apparently the same conditions as the flinty kernels, not merely in the same field, but on the same

plant or in the same head, indicates that yellow-berry is actually different from ordinary soft wheat.

Roberts and Freeman (*6*) suggested that heredity is a strong factor in determining the occurrence of yellow-berry in wheat and that pure varieties could probably be isolated that would produce little or no yellow-berry. To establish the correctness or incorrectness of this view a large number of pure strains of winter wheat were examined by the writer, and the percentage by weight of yellow-berry was determined in each.

The method pursued was as follows: From each strain of wheat, 100 cc. of grain were taken and weighed. The yellow-berry kernels were then separated and weighed. The starchy spots in the kernel almost invariably begin to appear around the germ or embryo—that is to say, at the lower end of the kernel as it stands in the glumes—and spread from there upward. In no case do the starchy spots begin to appear at the brush or tip end of the kernel. The area of the starchy spots may vary from minute dots to the entire grain. Since the opaque, starchy spots in a flinty, translucent wheat kernel may be large or small, the separation of the yellow-berry kernels must be made according to an arbitrary standard. It was decided to include as yellow-berry all kernels of which one-half or more of the grain was opaque and starchy. The starchy kernels were separated on this basis and weighed. The flinty kernels—those showing no opaque spots at all—were also separated and weighed, and the residue, if any, was designated as "neutral grains."

The separating and weighing of the kernels was done by two persons, designated in the table by their initials, "L" and "A," who by long experience became very expert in making the analyses of the samples. By having a part of the samples which were analyzed by one checked by the other it was found that very little difference resulted from the different individual judgments of "L," who did the earlier, and "A," who did the later work. It is therefore concluded that the percentage of error due to the personal equation is negligible, and that it is completely overshadowed by the positive differences in the samples themselves.

In all, 164 lots of wheat were studied, of which 77 were pure strains and 87 were checks or controls. The pure strains were grown in single rows alternately with the controls. All the rows were of the same length, 66 feet, and stood 8 inches apart. The variety used for the control rows was not a pure line but was, nevertheless, an unusually pure race of Kharkov, a standard variety long and successfully grown here. All the rows, whether of pure-line wheats or controls, contained 250 grains each, planted equidistant in the rows. The wheat was all grown in the same field, which was divided lengthwise into blocks, and each block into plots separated by narrow alleyways. The plots were all 100 by 100 feet in size. In Table I the rows are grouped according to dates of harvesting in 1908. The control row following each pure strain is given

the number of that same strain, followed by the letter "C" to indicate that it is a control. Thus, 1104 is followed by 1104 C. In cases where the numbering is not consecutive, as where a control of a different number follows a pure strain, or where no control at all is given after a pure strain, the reason lies in the fact that the omitted rows did not have the same harvesting date, or else, either through accident or winterkilling, they had been eliminated. The results of this study are given in Table I and summarized in Table II. The percentage of yellow-berry in each strain in 1907 is included in Table I so far as this information is available.

TABLE I.—*Yellow-berry in different strains and varieties of hard winter wheat*

Row No.	Date of harvest, 1908.	Plot No.	Percentage of yellow-berry.	
			1907.	1908.
1152 (A)	June 24	7	3.0	4.8
1149 (A)	do	7	17.0	17.3
1126 (L)	do	7	5.0	40.4
1125 (A)	do	7	1.0	33.8
1066 (A)	June 25	5	5.0	6.8
1068–4 (L)	do	5	.....	30.8
1068–5 (L)	do	5	49.0	34.0
1081–C (A)	do	5	.....	11.9
1122–C (A)	do	6	.....	27.6
1124–C (L)	do	6	.....	32.4
1125–C (A)	do	6	.....	32.8
1126–C (L)	do	6	.....	33.0
1128 (A)	do	6	9.0	24.1
1131 (L)	do	6	4.0	34.2
1135–C (L)	do	6	.....	28.2
1142–C (A)	do	7	.....	45.3
1145–C (L)	do	7	.....	32.8
1157 (A)	do	7	1.0	4.6
1157–C (A)	do	7	.....	6.8
1161 (A)	do	7	7.0	4.8
990 (L)	June 26	3	1.0	11.1
991 (L)	do	3	.....	18.0
1000 (L)	do	3	2.0	16.5
1003-C (L)	do	3	.....	19.5
1008 (A)	do	4	5.0	20.5
1008-C (A)	do	4	.....	26.2
1069 (A)	do	5	5.0	5.2
1074-C (A)	do	5	.....	21.2
1075 (L)	do	5	5.0	22.2
1080 (A)	do	5	5.0	1.8
1080-C (A)	do	5	.....	19.8
1081 (A)	do	5	5.0	15.8
1093-C (L)	do	5	.....	40.7
1094 (L)	do	5	.....	70.4
1094-C (L)	do	5	10.0	28.2
1095-C (L)	do	5	.....	22.6
1103 (A)	do	6	11.0	29.7
1098 (A)	June 27	6	.....	37.1
990-C	June 30	3	.....	17.9
991-C (L)	do	3	.....	27.0
994-C (L)	do	3	.....	21.0
1000-C (L)	do	3	.....	13.0
996-C (L)	July 1	3	.....	29.5

TABLE I.—*Yellow-berry in different strains and varieties of hard winter wheat*—Con.

Row No.	Date of harvest, 1908.	Plot No.	Percentage of yellow-berry.	
			1907.	1908.
1004-C (A)	July 1	3	.....	13.6
1035-C (A)	...do.....	4	.....	14.0
1036 (A)	...do.....	4	10.0	56.8
1036-C (A)	...do.....	4	.....	19.0
1038 (L)	...do.....	4	20.0	62.1
1038-C (L)	...do.....	4	.....	36.0
1058-1-C (L)	...do.....	4	.....	40.1
1059-3-C (L)	...do.....	4	.....	43.3
1059-4 (A)	...do.....	4	5.0	23.3
1059-4-C (A)	...do.....	4	.....	23.0
1064-C (A)	...do.....	4	.....	6.2
1066-C (A)	...do.....	5	.....	12.8
1068-C (L)	...do.....	5	.....	33.9
1068-5-C (L)	...do.....	5	.....	38.0
1069-C (A)	...do.....	5	.....	12.8
1070-C (A)	...do.....	5	.....	10.1
1071 (A)	...do.....	5	.....	16.2
1071-C (A)	...do.....	5	.....	24.6
1072 (L)	...do.....	5	.....	47.0
1072-C (L)	...do.....	5	1.0	44.2
1073 (A)	...do.....	5	.....	22.2
1073-C (A)	...do.....	5	11.0	13.4
1075-C (L)	...do.....	5	.....	49.7
1076 (A)	...do.....	5	.....	50.9
1077 (L)	...do.....	5	16.0	50.4
1077-C (L)	...do.....	5	28.0	44.5
1093 (L)	...do.....	5	.....	44.9
1076-C (A)	...do.....	5	4.0	18.8
1098-C (A)	...do.....	6	.....	20.2
1102 (A)	...do.....	6	21.0	45.8
1103-C (A)	...do.....	6	.....	15.0
1104 (A)	...do.....	6	10.0	16.7
1104-C (A)	...do.....	6	.....	11.9
1105-C (A)	...do.....	6	.....	28.9
1106 (A)	...do.....	6	9.0	14.8
1106-C (A)	...do.....	6	.....	32.3
1107 (A)	...do.....	6	3.0	26.8
1107-C (A)	...do.....	6	.....	19.0
1108 (A)	...do.....	6	1.0	37.2
1108-C (A)	...do.....	6	.....	16.4
1109 (A)	...do.....	6	2.0	37.4
1110 (A)	...do.....	6	2.0	50.9
1110-C (A)	...do.....	6	.....	25.5
1111-C (A)	...do.....	6	.....	27.0
1113 (A)	...do.....	6	.....	10.0
1113-C (A)	...do.....	6	.....	26.4
1114 (A)	...do.....	6	.....	9.6
1114-C (A)	...do.....	6	.....	31.2
1115 (A)	...do.....	6	1.0	29.8
1115-C (A)	...do.....	6	.....	32.3
1116 (A)	...do.....	6	7.0	40.5
1116-C (A)	...do.....	6	.....	32.9
1124 (L)	...do.....	6	2.0	16.5
1128-C (A)	...do.....	6	.....	16.9
1130 (A)	...do.....	6	.....	43.5
1130-C (A)	...do.....	6	.....	24.0
1132 (L)	...do.....	6	20.0	80.4

TABLE I.—*Yellow-berry in different strains and varieties of hard winter wheat*—Con.

Row No.	Date of harvest, 1908.	Plot No.	Percentage of yellow-berry.	
			1907.	1908.
1132–C (L)	July 1	6	.....	69.0
1135 (L)	...do.....	6	21.0	86.0
1140 (L)	...do.....	6	2.0	94.5
1140–C (L)	...do.....	6	.....	66.9
1145 (L)	...do.....	7	25.0	99.0
1150–C (A)	...do.....	7	.....	10.3
1151 (A)	...do.....	7	1.0	8.8
1151–C (A)	...do.....	7	.....	12.9
1152–C (A)	...do.....	7	.....	11.4
1154 (L)	...do.....	7	6.0	75.3
1154–C (L)	...do.....	7	.....	41.7
1160 (L)	...do.....	7	.....	49.0
1161–C (A)	...do.....	7	.....	10.3
1162 (A)	...do.....	7	5.0	62.4
1162–C (A)	...do.....	7	.....	11.4
1163 (L)	...do.....	7	12.0	69.1
1163–C (L)	...do.....	7	.....	28.3
1164–1 (L)	...do.....	7	23.0	45.3
1164–1–C (L)	...do.....	7	.....	34.6
1164–2 (L)	...do.....	7	30.0	64.8
1372–1 (A)	...do.....	7	2.0	40.3
1372–1–C (A)	...do.....	7	.....	15.2
1372–8–C (A)	...do.....	7	.....	14.1
1117–C (L)	...do.....	7	.....	25.8
1160 (L)	July 3	7	30.0	61.5
1164–2–C (L)	...do.....	7	.....	52.6
1058–6 (L)	...do.....	8	.....	26.7
1058–6–C (L)	...do.....	8	.....	29.2
1117 (L)	...do.....	8	.....	53.0
1138–C (A)	...do.....	8	.....	8.1
1059–7 (L)	...do.....	1	.....	45.4
1059–7–C (L)	...do.....	1	.....	45.2
1067 (L)	...do.....	1	17.0	87.0
1067–C (L)	...do.....	1	.....	41.5
1091 (L)	...do.....	1	.....	90.2
1091–C (L)	...do.....	1	.....	43.4
1146 (L)	...do.....	1	32.0	90.4
1146–C (L)	...do.....	1	.....	59.7
1147–C (L)	...do.....	1	.....	78.4
1003 (L)	July 6...	3	10.0	15.0
1004 (A)	...do.....	3	.....	59.8
1058–1 (L)	...do.....	4	.....	32.5
1058–5 (A)	...do.....	4	1.0	20.3
1059–3 (L)	...do.....	4	1.0	38.9
1059–6 (L)	July 10	1	.....	30.0
1059–6–C (L)	...do.....	1	.....	38.0
1002–C (A)	...do.....	1	.....	33.7
1039 (L)	...do.....	1	.....	67.9
1039–C (L)	...do.....	1	.....	63.0
1068–1–C (A)	...do.....	1	.....	26.4
1096–C (A)	...do.....	1	.....	28.3
1139 (L)	...do.....	1	.....	92.2
1139–C (L)	...do.....	1	.....	79.5
1147 (L)	...do.....	1	4.0	94.4
1158 (L)	July 12	7	1.0	65.5
1158–C (L)	...do.....	7	.....	38.8

TABLE II.—*Summary of yellow-berry in hard winter wheat, 1908*

Date of harvesting, 1908.	Total number of cases.	Number of pure strains.	Percentage of yellow-berry.	Number of controls.	Percentage of yellow-berry.	Average percentage of yellow-berry.
June 25	16	7	20	9	28	24
26	17	10	21	7	25	23
30	4	0	.....	4	20	20
July 1	82	33	45	49	26	34
3	15	7	65	8	45	54
6	5	5	33	0	.....	33
10	10	4	71	6	45	55

Tables I and II give the results for 73 pure strains and 83 controls out of the total number of 77 and 87, respectively, that were originally planted. The average percentage of yellow-berry in the control plots is given in Table III.

TABLE III.—*Average percentage of yellow-berry in control plots, 1908*

Plot No.	Number of rows.	Percentage of yellow-berry.
3	7	20.2
4	8	26.0
5	17	26.3
6	22	29.5
7	15	24.5
8	3	21.0
Average	.....	26.1

From this table it appears that the control rows were quite constant in their tendency to produce yellow-berry. However, as shown in figure 1, there is considerable variation in the amount of yellow-berry in individual rows. The general trend of the percentage of yellow-berry in the pure strains follows that of the controls. This indicates that the differences depend upon the same causes in the pure strains as in the controls, and that the changing conditions in different parts of the plot had more influence in causing an increase or a decrease in yellow-berry than did any hereditary factors.

The yield per row presents a similar phenomenon. There is a greater variability in the yield of the individual rows in the pure strains than in the control rows, but the general upward and downward trend of the two curves coincides very closely. This would indicate that external conditions in the plots were more important than varietal characteristics in determining both the yield and the percentage of yellow-berry.

An inspection of Table I shows that there are a number of cases in which there is apparent coincidence between the percentage of yellow-berry produced in one season and the percentage produced by the same strain the following season. However, a correlation table between yellow-berry percentages for the two successive seasons plotted for 56 strains that were planted and harvested on the same date gives a correlation coefficient of only 0.078 ± 0.005. This extremely low correlation indicates that the external conditions are the determining factors to a degree which the hereditary tendencies of the plant have little power to modify. On the other hand, a graph showing the percentage of yellow-berry in 1907 and 1908 for those strains having 10 per cent or more of yellow-berry indicates that there is a hereditary relation; and were the number of cases larger, distinct indications of inheritance would be seen.

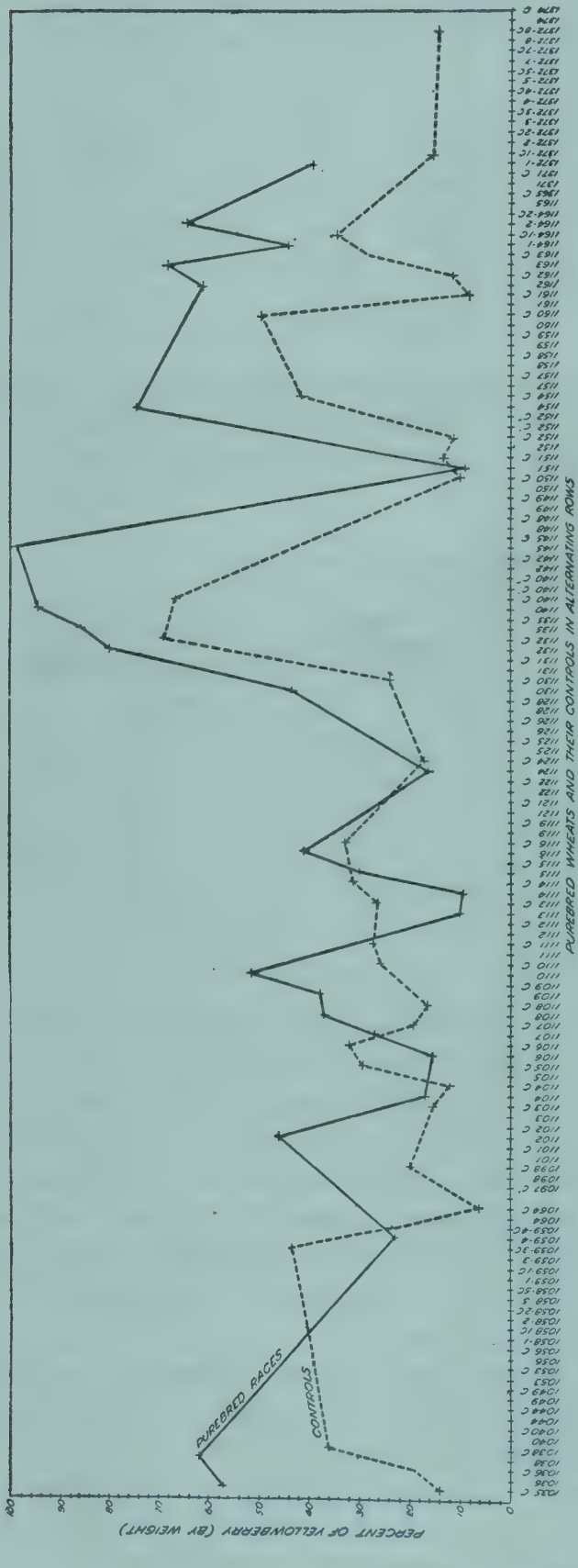

FIG. 1.—Percentage by weight of yellow-berry kernels in pure-bred wheats and their controls, 1908.

The relation between date of harvesting and the percentage of yellow-berry is indicated in figure 2. It is evident that from June 25 to July 1 there was an increase in the percentage of yellow-berry. The total number of rows harvested July 3 and 10 are not sufficient to permit definite conclusions with respect to these dates. It appears, however, that in the season of 1908 there was a close relation between the percentages of

yellow-berry and the date of ripening. This is in harmony with the general assumption that a longer growing and a slower ripening period produces yellow-berry.

PHYSICAL CHARACTERS OF YELLOW-BERRY

Lyon and Keyser (*5, p. 32*) cite Nowacki to the effect that—

the difference between mealy and horny wheat kernels is due to the presence in the former of a larger volume of air spaces than in the latter. He urges that the vacuoles

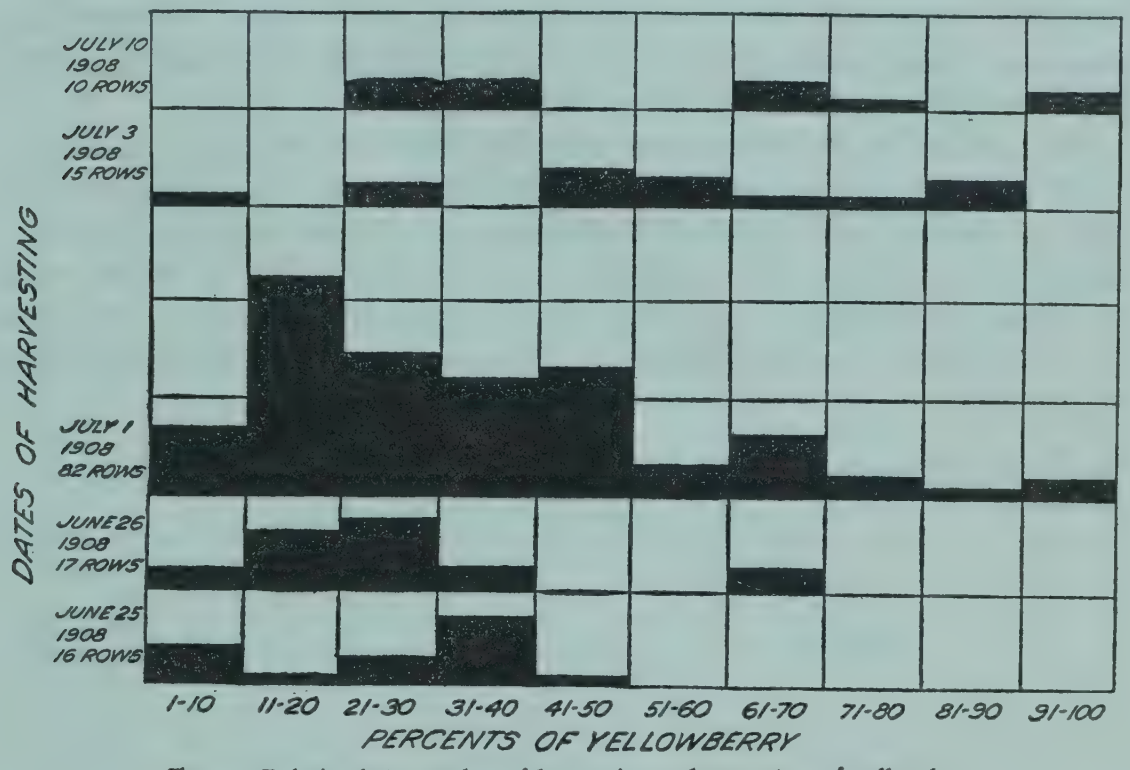

FIG. 2.—Relation between date of harvesting and percentage of yellow-berry.

that occur in the protoplasm of the cell decrease in size and number as the endosperm develops, and that the more protoplasm, the smaller and fewer the vacuoles.

Lyon and Keyser (*5, p. 34*) found that—

a typical mealy wheat like the soft, white Sonora of California contained starchy granules measuring from 0.02817 millimeters in diameter for the larger, to 0.005634 millimeters for the smaller. A typical horny Turkish Red kernel contained starch grains varying between the extremes of 0.014085 and 0.002817 millimeters in diameter. A typical yellow-berry Turkish Red kernel showed larger starch granules, 0.017042 millimeters for the larger, and 0.003081 millimeters for the smaller sizes.

Cobb (*3, p. 512*) found that—

it is noticeable that when the grain is rich in nitrogenous matter the number of large starch granules is smaller. As we pass in such grains in our examination from the center to the outside, we note a gradual decrease in the size of the starch granules, and even at some little distance from the aleuron layer the cells are filled with small granules only.

Lyon and Keyser's examination (*5, p. 35*) of horny and starchy kernels revealed more numerous and larger vacuoles in the latter, with only an

occasional vacuole in the former. It is stated that large starch granules and large or numerous vacuoles are associated in starchy kernels, and that—

> the difference in structure between the horny and the yellow kernels is also accompanied by a difference in composition, the yellow kernels containing less nitrogen.

The size of the starch granules in yellow-berry and in hard, flinty wheat was studied by the writer. Yellow-berry kernels were taken from a number of pure wheats, and samples of the opaque or yellow portions of the endosperm were removed from these by means of a dental drill. Samples were taken from 10 kernels to get a fair average. Similar samples were taken from the horny or flinty portion of the same 10 kernels, the drill being burned off each time after use.

The yellow-berry endosperm samples were shaken up in alcohol, stained with iodin in potassium iodid, and mounted for measurement with a Bausch and Lomb filar micrometer. Five hundred measurements were made for each 10-grain sample of each strain of wheat used. In all cases, the largest starch granules visible in any given field were the ones chosen for measurement. The results of this study are given in Table IV.

TABLE IV.—*Measurements of starch grains from yellow-berry and from hard kernels of 10 pure lines of wheat*

Sample No.	Measurement of starch granules in—		Difference between soft and hard grains.
	Hard grains.	Soft grains.	
	Mm.	*Mm.*	*Mm.*
951	0.030726	0.024819	−0.005907
1094	.024999	.029095	+ .004096
1119	.025910	.023940	− .001970
1126	.027315	.024977	− .002338
1150	.032587	.027981	− .004606
1516–8	.027873	.028878	+ .001005
1592–6	.028234	.028969	+ .000735
1687–4	.029413	.027965	− .001448
1687–8	.031869	.023822	− .008047
1687–10	.033200	.028519	− .004681
Average	.029212	.026897	.002315

These results show that in 7 out of 10 cases the average diameter of the starch grains in the hard portions of the kernels was greater than in the soft or yellow-berry portions, while in 3 cases it was less. These results are exactly the reverse of those obtained by Lyon and Keyser (*5, p. 23–26*).

The writer is unable to account for the discrepancy in the two sets of data. It would seem, however, that since in the present case an average of 500 measurements was taken and the largest starch grains in each

microscopic field were measured, fairly uniform and accurate results have been obtained.

Table V shows the frequency of distribution of the starch grains with respect to size, expressed in micromillimeters (microns) in nine of the races or pure strains just considered. From this table it appears that, in both the hard kernels and the yellow grains, the greatest number of individual cases (the mode) falls into the class of 25 to 29.9 micromillimeters diameter, although the average size of the starch grains in the hard kernels was about 2.3 microns greater than in the yellow-berry kernels.

TABLE V.—*Distribution of starch grains of yellow-berry and hard kernels with respect to size*

[Diameter of starch grains expressed in micromillimeters $\left(\frac{mm.}{1000}\right)$]

Pedigree No.	Number of starch grains having diameter of—												
	14.9	19.9	24.9	29.9	34.9	39.9	44.9	49.9	54.9	59.9	64.9	69.9	74.9
951 h	....	26	118	121	90	78	31	22	14	....	....	....	....
951 y	....	46	235	172	40	5	1	....	....	....	....	....	....
1094 h	1	63	207	167	46	16	7	1	....	....	....	....	....
1094 y	....	9	87	198	154	45	7	....	....	....	....	....	....
1119 h	....	36	197	174	74	19	....	....	....	....	....	....	....
1119 y	....	69	259	136	33	2	1	....	....	....	....	....	....
1126 h	....	7	133	245	97	18	3	....	....	....	....	....	....
1126 y	....	46	209	196	46	3	....	....	....	....	....	....	....
1516–8 h	....	5	124	224	118	26	3	....	....	....	....	....	....
1516–8 y	....	6	72	218	180	23	1	....	....	....	....	....	....
1592–6 h	....	15	127	179	124	47	....	....	....	....	....	....	....
1592–6 y	....	6	71	227	162	29	5	....	....	....	....	....	....
1687–4 h	5	32	88	147	117	68	22	8	2	0	1	....	....
1687–4 y	5	46	119	166	90	14	23	6	2	....	....	....	....
1687–8 h	17	42	57	93	83	71	79	23	5	2	1	1	1
1687–8 y	2	115	220	127	46	13	2	....	....	....	....	....	....
1687–10 h	9	27	64	99	108	83	51	25	19	7	3	3	2
1687–10 y	10	39	104	150	108	61	20	6	2	....	....	....	....
Total, hard	32	253	1,125	1,449	857	426	193	79	40	9	5	4	3
Total, yellow	17	382	1,376	1,590	859	224	60	12	4	....	....	....	....

Other physical characteristics of the yellow-berry and hard kernels—for example, specific gravity, average kernel-weight, and volume-weight, are given in Table VI for 10 strains of wheat used for the study of the size of starch granules. The volume-weight, the test weight per bushel, and the average weight per kernel is higher for the yellow-berry than for the hard kernels. The specific gravity is somewhat higher in the hard wheat. These results agree in general with those previously reported by the writer (*6*), except that in the earlier investigation the average weight per kernel was higher for the hard wheat.

Snyder (*7*, *8*) has investigated the comparative weight of light and dark seeds taken from the same samples of varieties from 31 miscellaneous

sources, and of 32 varieties grown from selected seed. Most of these were Minnesota-grown. The average weight per kernel was higher for the dark grains in one case and for the light grains in the other.

TABLE VI.—*Specific gravity, kernel-weight, and volume-weight of hard (h) and yellow-berry (y) wheat*

Sample No.	Specific gravity.	Average weight per kernel.	Volume weight.	Test weight.
		Gm.	*Gm. per 100 cc.*	*Pounds.*
951 y	1.368			
	1.367	0.029	83.42	64.76
951 h	1.387			
	1.378	.027	80.85	62.80
1094 y	1.351			
	1.362	.031	80.00	62.15
1094 h	1.395			
	1.385	.027	79.63	61.86
1119 y	1.380			
	1.373			
1119 h	1.399			
	1.399	.031	79.00	61.37
1126 y	1.388			
	1.360	.025	83.72	64.59
1126 h	1.406			
	1.411	.028	82.22	63.44
1150 y	1.379			
	1.379	.033	79.12	61.46
1150 h	1.376			
	1.376	.033	77.12	60.33
1516–8 y	1.370			
	1.367	.029	82.88	63.95
1516–8 h	1.387			
	1.390	.031	78.66	60.69
1592–6 y	1.345			
	1.347	.026	77.04	59.44
1592–6 h	1.368			
	1.381	.022	77.23	59.59
1687–4 y	1.378			
	1.370	.030	82.05	63.31
1687–4 h	1.404			
	1.404	.032	79.29	61.18
1687–8 y	1.372			
	1.378	.029	79.40	61.68
1687–8 h	1.396			
	1.395	.029	78.88	61.28
1687–10 y	1.377			
	1.370	.028	80.00	62.15
1687–10 h	1.391			
	1.386	.027	75.15	58.38
Average, y	1.369	.0291	80.66	62.49
Average, h	1.392	.0287	78.73	61.00

Stewart and Hirst (*10*) and Stewart and Greaves (*9*), of the Utah Agricultural Experiment Station, found in comparing the average weight of kernels of a considerable number of hard winter wheats, semihard winter wheats, and soft winter wheats, that soft wheat varieties had the heaviest kernels and hard wheats the lightest.

THE CHEMICAL COMPOSITION OF YELLOW-BERRY WHEAT

The chemical composition of yellow-berry, especially as related to protein content, has been the subject of several investigations. Snyder, of the Minnesota Experiment Station (*7*, *8*), in comparing 63 light (starchy) and 30 dark (flinty) lots of grain, found a slight difference in the protein content in favor of the hard grain. The chief differences in chemical composition of yellow-berry and hard kernels of the same sample found by the writer are a higher moisture content, a lower protein content, and a higher starch content in the yellow-berry kernels as compared with the hard kernels. The data secured in this investigation are given in Tables VII and VIII.

TABLE VII.—*Analyses of yellow-berry wheat*

Chemistry laboratory No.	Botany laboratory No.	Moisture.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Pentosans.	Starch.	Ether extract.
		Per cent	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
603	951	8.47	1.83	10.36	2.51	74.99	7.60	69.34	1.84
605	1094	8.42	1.86	10.48	2.22	75.08	7.66	67.65	1.94
607	1119	8.59	1.71	10.44	2.19	75.29	7.27	64.50	1.78
609	1126	7.37	1.64	9.72	2.10	77.04	7.68	67.33	2.13
611	1150	7.72	1.96	10.76	2.05	75.61	7.99	66.17	1.90
613	1516–8	7.71	1.57	10.80	2.19	75.68	7.08	70.62	2.05
615	1592–6	7.73	1.71	10.04	2.53	76.04	8.03	63.47	1.95
617	1687–4	7.21	1.87	10.80	2.15	75.92	7.85	63.58	2.05
619	1687–8	6.71	1.94	10.79	2.30	76.16	7.73	69.26	2.10
621	1687–10	8.07	1.79	10.96	2.22	74.96	7.67	68.18	2.00
Total		78.46	17.88	105.15	22.46	756.77	75.56	670.10	19.74
Average		7.846	1.788	10.515	2.246	75.677	7.556	67.01	1.974

TABLE VIII.—*Analyses of hard, flinty wheat*

Chemistry laboratory No.	Botany laboratory No.	Moisture.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Pentosans.	Starch.	Ether extract.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
604	951	8.20	1.99	12.00	2.65	73.31	8.17	68.82	1.85
606	1094	8.63	2.02	12.00	2.50	73.02	7.86	60.92	1.83
608	1119	7.97	2.06	12.00	2.36	73.96	7.32	66.95	1.65
610	1126	7.41	1.91	11.32	2.33	75.10	7.87	63.96	1.93
612	1150	7.68	2.03	12.30	2.11	74.06	8.37	64.60	1.82
614	1516–8	7.22	1.87	12.08	2.18	74.55	7.85	65.79	2.10
616	1592–6	7.20	1.90	12.04	2.50	74.51	8.52	62.01	1.85
618	1687–4	6.98	1.82	12.12	2.19	74.96	7.66	62.09	1.95
620	1687–8	7.24	2.02	12.08	2.35	74.34	7.58	65.67	1.97
622	1687–10	7.83	2.04	11.96	2.49	73.80	7.63	66.81	1.88
Total		76.34	19.66	119.90	23.66	741.61	78.83	647.62	18.83
Average		7.634	1.966	11.99	2.366	74.161	7.883	64.762	1.883

The results show a higher percentage of starch in the yellow-berry wheat than in the flinty kernels, the average percentages being 67.01 and 64.762, respectively. They show also an average starch ratio of 6.37 for the yellow-berry kernels and of only 5.40 for the flinty kernels. It appears probable that the smaller amount of protein in the starchy grains is not only fully compensated for by an equivalent deposition of starch but more than compensated for, since the percentage of protein is 1.475 less and the percentage of starch 2.248 greater in the starchy than in the flinty kernels.

SUMMARY

(1) This investigation is a continuation of the work reported in Kansas Agricultural Experiment Station Bulletin 156.

(2) The opaque, starchy spots in wheat kernels which give them the designation of yellow-berry kernels almost invariably begin to appear in the neighborhood of the germ or embryo, the lower end of the kernel as it stands on the plant, and spread from there upward.

(3) One hundred and sixty-four lots of wheat were investigated to determine the relation of yellow-berry to field conditions, especially the period between first heading and ripening. Seventy-seven of these lots were pure strains or pure lines, and 87 were checks or controls.

(4) In determining the percentage of yellow-berry, an arbitrary standard was adopted. If one-half or more of a kernel was opaque it was weighed as a yellow-berry kernel. The flinty kernels free from opaque portions were weighed separately, and the residue of the kernels were designated as neutral grains.

(5) The variation in yellow-berry percentages in the yields of the control rows was closely followed by that of the pure-line rows alternating with them. The general trend of the whole series of the pure lines follows that of the controls.

(6) The conclusion from the field tests is that the operation of common causes for the production of yellow-berry overshadowed any differences that may have been due to hereditary tendencies, and precludes a definite statement regarding the relation of hereditary tendencies in hard winter wheats toward the production of yellow-berry. That some isolated pure strains of wheat are freer from yellow-berry than others growing in the same field and under apparently identical conditions of soil and climate is, however, possible.

(7) With respect to the relation of yellow-berry to date of ripening, the experiment shows a higher percentage of yellow-berry with the later dates of ripening.

(8) The comparative size of the starch granules in yellow-berry and in flinty grains was investigated, 500 measurements of starch grains being made from hard and from yellow-berry samples of 10 strains of of pure-line wheats. The largest starch grains in the yellow-berry portions of the kernel were found to be smaller on the average than the largest starch grains in the flinty portions of the same kernels. These results seem to contradict those of Cobb and of Lyon and Keyser.

(9) In respect to the average kernel-weight, the yellow-berry kernels were found to weigh on the average 0.4 mg. more than the flinty kernels, based on the average weight (air-dried at 100° C.) of 100 kernels. In an earlier study the flinty kernels were on the average 1.4 mg. heavier.

(10) In specific gravity the flinty kernels were found to be 0.0230 heavier than the yellow-berry kernels.

(11) The yellow-berry kernels were found to be higher in moisture and starch content and lower in protein and ash than the hard, flinty kernels.

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NOTE ON THE EUROPEAN CORN BORER (PYRAUSTA NUBILALIS HÜBNER) AND ITS NEAREST AMERICAN ALLIES, WITH DESCRIPTION OF LARVÆ, PUPÆ, AND ONE NEW SPECIES

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The introduction of the European corn borer (*Pyrausta nubilalis* Hübner) into certain sections of Massachusetts and New York and the possibility of its wider distribution has necessitated a careful study of the larva of this dangerous pest, particularly as the larva of a native Pyrausta also attacks corn, has much the same habits as that of *P. nubilalis*, and so closely resembles it that the two are easily confused. The adult females of the two species are also very similar, and to any but a specialist familiar with the group they are certain to cause difficulty. For this reason it is desirable to have full descriptions of adults, pupæ, and larvæ which will enable positive identification and will separate *P. nubilalis* from its nearest American allies. The present paper is presented with this object.[2]

PYRAUSTA

GENERAL CHARACTERS

ADULT (PL. 7, A, B, E, F; 8, B, C)

Ocelli present. Proboscis developed. Labial palpi developed; porrect; triangularly scaled; third joint hidden by hair. Maxillary palpi present; slightly dilated at apex. Frons rounded. Antennæ three-fourths; finely ciliated. Tibiæ smooth-scaled; outer spurs short; outer medial spur not more than two-thirds the length of the inner. Forewing with 12 veins; 1c absent; 1a separate from 1b; 1b simple; 2 from before angle of cell; 3, 4, 5 from lower angle of cell; 6 from near upper angle of cell; 7 from the cell, to termen, almost straight; 8 and 9 stalked; 10 closely approximate with 8 and 9. Hindwing as broad as forewing; frenulum present, single in male, multiple in female; median vein nonpectinate on upper side; 8 veins; 1a,

[1] For material necessary for these studies the writer is indebted to Messrs. W. R. Walton, D. J. Caffrey, and Geo. G. Ainslie, of the Bureau of Entomology, especially to the latter, who has furnished reared series of moths of *P. ainsliei* and *P. penitalis* and a quantity of larvæ and pupæ of both species. In the United States National Museum, aside from this, there are bred series of *P. penitalis* and *P. ainsliei* from various localities.

Dr. L. O. Howard has also kindly provided authentic European larvæ, blown and alcoholic, of *P. nubilalis*, secured through the courtesy of Prof. F. L. Bouvier, of Paris, France.

For the drawings accompanying this paper the writer is indebted to Miss E. Hart and Miss Ada F. Kneale, of the Bureau of Entomology. Miss Kneale has contributed figures of the female genitalia on Plate 8. The rest are by Miss Hart.

[2] Since these studies were begun, a suggestive paper dealing with the larvæ of *P. nubilalis* and other lepidopterous borers has appeared. (MOSHER, Edna. NOTES ON LEPIDOPTEROUS BORERS FOUND IN PLANTS, WITH SPECIAL REFERENCE TO THE EUROPEAN CORN BORER. *In* Jour. Econ. Ent., v. 12, no. 3, p. 258–268. 1919.)

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1b, 1c present; 1b simple; 3, 4, 5 from lower angle of cell; 6, 7 from upper angle; 7 anastomosing with 8 beyond cell. Male genitalia with uncus rudimentary or absent; transtilla present; harpes with prominently developed clasper.

PUPA (PL. 9, C-F)

Moderately slender; abdominal segments gradually tapering; smooth except for a slight rugosity on dorsum and a single row of 4 or 5 short spines on dorsum of abdominal segments 1 to 7; wings extending to or nearly to ventro-caudal margin of fourth abdominal segment; cephalic end bluntly rounded, tapering from mesothorax; epicranial suture present, represented by a straight line; vertex distinct, rather narrow; labrum, pilifers, and maxillary palpi well developed; labial palpi small; prothoracic and mesothoracic legs not extending cephalad between sculptured eyepiece and antenna; maxillæ long, extending nearly the length of the wings; femora of prothoracic legs clearly indicated; prothoracic legs extending half the length of the wings; mesothoracic and metathoracic legs extending to the tips of the wings; antennæ extending nearly the length of the wing; proleg scars plainly visible on abdominal segments 5 and 6; mesothoracic spiracle with a strongly chitinized caudal ridge, without setæ; abdominal spiracle slightly produced; anal and genital openings slitlike in both sexes; cremaster present, prominent, stout, spatulate, and armed at extremity with a cluster of 4 or 5 short curled hooks.

LARVA (PL. 10, A-D; 11, A, D-H)

Cylindrical; moderately stout; abruptly tapering at caudal end. No secondary hair. Legs and prolegs normal. Crochets triordinal, in a circle broken outwardly. No anal fork. Prothoracic shield moderately broad, divided. Spiracles oval, moderate; that on eighth abdominal segment slightly higher than those on abdominal segments 1 to 7; no more than 1½ times as large; same size as that on prothorax. Skin covered with fine granulations (Pl. 11, E, F) especially strong and dense on dorsum, diminishing toward venter and absent in folds marking the body areas and a small space about the chitinized tubercles.

Body setæ moderately long; tubercles prominent, broadly chitinized; IV and V on abdominal segments 1 to 8 under the spiracle and approximate; prespiracular shield of prothorax small or moderate, nearly square, bearing only two setæ (IV and V) situated ventro-cephalad of the spiracle, III of prothorax absent; group VI bisetose on prothorax, unisetose on mesothorax and metathorax; IV and V united on abdominal segment 9 and approximate to III; III directly in front of the spiracle on abdominal segment 8, over the spiracle on abdominal segments 1 to 7; III^a present; group VII trisetose on abdominal segments 1 to 6, bisetose on abdominal segment 7, unisetose on abdominal segments 8 and 9; abdominal segment 9 with all setæ in a vertical line, 1 absent; on abdominal segments 1 to 8, II is latero-caudad of I; prothorax with II^a higher than I^a, dorso-caudad and remote from II^b, closer to I^a than to II^b, II^b on the level of puncture z; I^b equidistant from I^c and puncture z, punctures x and y dorso-caudad of I^a, distance between I^c and II^c slightly greater than between I^b and I^c.

Head capsule spherical, nearly square in outline viewed from above, slightly wider than long; greatest width at middle of head; incision of dorsal hind margin not over one-fourth the width of the head; distance between dorsal extremities of hind margin less than one-half the width of the head; from dorsum of antennal ring a slight projection of the epicranium forming an antennal shield (ATS). Frons broad, as long as or a trifle longer than wide, reaching beyond middle of head. Adfrontal sutures extending to incision of dorsal hind margin. Longitudinal ridge (LR) short, less than one-half the length of the frons.

Ocelli six; lenses well defined.

Epistoma normal.

Frontal punctures close together; very slightly forward of frontal setæ; distance between punctures less than distance from puncture F^a to setæ F^1; distance from frontal setæ (F^1) to first adfrontal seta (Adf^1) about equal to distance separating adfrontal setæ (Adf^1 and Adf^2); Adf^2 approximate to beginning of longitudinal ridge; puncture Adf^a about equidistant from Adf^1 and Adf^2.[1]

Epicranium with the normal number of primary setæ and punctures and with the three ultraposterior setæ and one ultraposterior puncture distinguishable. Anterior setæ (A^1, A^2, A^3) forming a slightly obtuse angle; anterior puncture (A^a) posterior to seta A^2. Posterior setæ (P^1 and P^2) and puncture P^b about middle of head; P^1 nearly on the level of lateral seta (L^1), behind the level of Adf^1; P^2 behind the level of place of juncture of adfrontal ridges; posterior puncture P^a approximate to lateral seta (L^1); posterior puncture P^b lying between P^1 and P^2 approximate to P^2; P^1, P^b, P^2 and setæ and puncture of ultraposterior group forming nearly a straight line with frontal seta (F^1). Lateral seta (L^1) well forward on head but not closely approximate to A^3; lateral puncture posterior or postero-ventrad to L^1, remote. Ocellar setæ (O^1, O^2, O^3) well separated; O^1 ventrad of ocelli II and III, approximate to ocellus III; O^2 ventrad or postero-ventrad of ocellus I; O^3 directly ventrad of O^2, remote, further from O^2 than O^2 is from O^1; ocellar puncture (O^a) approximate to ocellus VI. Subocellar setæ (SO^1, SO^2, SO^3) triangularly placed; puncture SO^a nearer to SO^2 and SO^3 than to SO^1. Genal puncture (G^a) anterior to the seta (G^1).

Labrum with median incision broadly triangular, moderately deep; median setæ (M^1, M^2, M^3) triangularly placed; M^2 postero-laterad of M^1 and considerably closer to M^1 than to M^3; La^1 directly laterad of and closely approximate to La^2; La^1 and La^2 on the level of M^1; La^3 and M^3 on the same level, rather well back of anterior margin of labrum ; puncture approximate and posterior to M^2.

Epipharyngeal shield narrowly bordering the greater part of median incision of labrum. Epipharyngeal setæ triangularly grouped; well separated and well behind anterior margin of epipharynx; narrow, moderately long. Epipharyngeal rods indicated only by their prominent posterior projections.

Maxillulæ normal; the large lateral lobes heavily spined but without blades or distinctly modified setæ.

PYRAUSTA NUBILALIS

Pyrausta nubilalis Hübner, 1901, *in* Staud. and Rebel, Cat. Lepidop., Aufl. 3, Bd. 2, p. 65, No. 1218.

ADULT

Male.—Underside of palpi snow white; palpi otherwise grayish fuscous. Head and thorax grayish fuscous. Forewing dark grayish fuscous; transverse antemedial and transverse postmedial lines outwardly margined with bright ochreous which, in the latter, broadens out to a distinct blotch at tornus; area between obicular and discal mark bright ochreous; at base of inner margin a distinct oval patch of firmly attached semimetallic brown sex scaling under surface scaling of the wings. Hindwing dark grayish fuscous; a broad, pale ochreous postmedian fascia not extending completely to dorsum. Abdomen dark grayish fuscous above; posterior margins of segments edged with a fine line of white scales. Genitalia (Pl. 7, A) as figured; apex of tegumen shortly trifurcate; anellus with two long, slender, dorsally projecting arms (anellus lobes); harpe with three stout spines arising from inner margin of sacculus at fusion with base of clasper; face of clasper oval, somewhat kidney-shaped. Alar expanse, 20 to 26 mm.

[1] There are some individual variations and considerable asymmetry in different specimens of the same species in the position of the adfrontal setæ and puncture and also in the length of the longitudinal ridge. The setæ will not always be on the same level on both sides of the head, and in some specimens Adf^a will be slightly nearer to Adf^1 than to Adf^2; but aside from such individual variations, which are common among lepidopterous larvæ and for which allowance must always be made, the characters hold remarkably well.

FEMALE.—Darker portion of palpi, head, and thorax pale or dull creamy ochreous. On forewing outer margins of transverse antemedial and transverse postmedial lines, terminal margin, and area between obicular and discal mark whitish ochreous. Postmedian fascia of hindwing whitish ochreous. Darker portions of forewing and hindwing pale gray, grayish ochreous, or ochreous gray tinged with ferruginous. Genitalia as figured (Pl. 8, A, B); genital opening without strong chitinous anterior margin; chitinized plate, posterior to genital opening, well developed, nearly square. Alar expanse, 18 to 34 mm.

PUPA

Fourteen to 16 mm. long; yellowish brown, darker towards extremities, cephalic end blackish brown; thorax but slightly humped; abdominal spiracles small, oval. Edges strongly chitinized, blackish brown; front smooth; cremaster (Pl. 9, F) longer than broad.

LARVA

Full grown 23 to 25 mm. long by 3 to 3.5 mm. broad. Body sordid white, shading to smoky fuscous on heavily granulose dorsal and lateral areas; smoky color forming a distinct, broad, longitudinal band along entire dorsum with a more distinct and darker narrow central band; creases of folds and areas immediately surrounding chitinized tubercles clear white; above and behind abdominal seta III and before abdominal seta I some of the muscle attachments are indicated by lines or clusters of white spots more or less fused.[1]

Chitinized areas of the body strongly pigmented; thoracic shield light yellow, laterally and caudally bordered by a narrow band of smoky fuscous and more or less spotted with brownish, above seta II^{c} the spots fusing into one or two more or less extended and conspicuous splotches; anal shield yellow, irregularly spotted, especially near margins, with smoky fuscous; chitinized areas of tubercles moderately large, irregularly oval or circular, yellow with a more or less extended border of smoky fuscous, which sometimes on those above the spiracle covers the entire tubercle; tubercle I of abdomen with one or two fuscous spots cephalo-laterad of the seta; dorsocaudad of the spiracle on proleg bearing abdominal segments 3 to 6 a small, chitinized, brownish, thornlike projection (Pl. 11, G, mt), quite plain in some specimens.[2] Setæ brownish at base, pale towards tip, slender. Thoracic legs yellow; claws brown. Crochets of prolegs unevenly triordinal; 32 to 46 (averaging 40); brown. Spiracles broadly oval; chitinous ring light brown.

Head brown, more or less mottled with blackish, in some specimens giving the whole head a blackish brown appearance; ocellar pigment black and in the form of a band under the ocelli, continuous. Anterior setæ A^1 and A^2 and puncture A^a in a line or with A^a a trifle postero-laterad of A^2, not postero-dorsad; A^2 somewhat nearer to A^1 than to A^3, A^1, A^2, and A^3 forming a decided obtuse angle; ocellar puncture O^a closely approximate and directly posterior to ocellus VI. Labium and maxillæ as figured; no decided hump in shoulder of stipes maxillaris; chitinized area of palpiger maxillaris yellow, strongly shaded with black. Mandible five-toothed; nearly square; distal tooth small and pointed; median edge outwardly angulated.

[1] These are the clear spaces referred to by Miss Mosher (OP. CIT.) and one of the characters which she uses to distinguish *Pyrausta nubilalis* from the so-called *P. penitalis* (*ainsliei*). The writer has been unable to find any real, consistent difference in this character between the two species. In some forms (particularly certain Phycitinae—Dioryctria and Pinipestis, for example) the points of attachment of the muscles are pigmented and slightly chitinized, forming a series of dark-colored pits, which are quite characteristic. Here only a few of the attachments are indicated by pits or spots, and these are colorless and more or less lost in the clear spaces of the folds indicating the limits of the body areas.

[2] Miss Mosher (OP. CIT.) refers to this structure as a sensory pore. It is in fact merely a chitinous support at the point of attachment of one of the strong proleg muscles and similar to the chitinization in the center of the proleg itself.

PYRAUSTA AINSLIEI

Pyrausta ainsliei n. sp.

Pyrausta penitalis Authores (nec. Grote).

Underside of palpi near base snow white; palpi otherwise yellow. Head and thorax yellow. Forewings pale yellowish with very slight dusting of darker cream yellow without the distinctly ferruginous powdering of *P. penitalis;* transverse antemedial and transverse postmedial lines as in *P. penitalis;* darker shading beyond transverse postmedial line faint; obicular marking as in *P. penitalis;* the dusky blotch beyond the cell reduced to a mere shading, scarcely distinguishable; terminal margin and cilia pale yellow; no sex scaling at base of inner margin of forewing of male. Hindwing as in *P. penitalis* except more distinctly marked than the pale forms of the latter species and lacking the ferruginous-ochreous margins of the small dark *P. penitalis.* Male genitalia as figured (Pl. 7, C); apex of tegumen rounded; anellus with two long, slender, dorsally projecting arms (anellus lobes); harpe with two or three stout spines arising from inner margin of sacculus at fusion with base of clasper; face of clasper triangular. Female genitalia as figured (Pl. 8, E, F), with genital opening strongly chitinized anteriorly. Alar expanse, 20 to 27 mm.

HABITAT.—Knoxville, Tenn., type locality (Ainslie and Cartwright); Arlington and Woodburn, Mass. (D. J. Caffrey); Milford, Conn. (M. P. Zapp); Hopewell Junction, N. Y.; Oak Station, Pa. (Fred Marloff); Plummer's Island, Md. (R. P. Currie); Tryon N. C. (W. F. Fiske); Missouri; Maine; St. Johns, Quebec (W. Chagnon).

FOOD PLANTS—Polygonum, Ambrosia, Xanthium, Eupatorium, corn.

TYPE—Cat. No. 22544, U. S. N. M.

P. ainsliei was described from one male type and eight male and seven female paratypes. It was named in honor of George G. Ainslie, of the Bureau of Entomology, who has made a special study of the life history and to whom the author is indebted for material and information on its habits. This is the species that has appeared in our collections and literature under the name of *P. penitalis* Grote. In our catalogues *P. nelumbialis* Smith is listed as a synonym. Upon examination of the genitalia of the specimens in the United States National Museum it became plain to the author that two distinct species were involved. There is a large series reared from Nelumbo from various sections of the United States. This is one species and differs markedly in adult and larva from the material reared from corn and Polygonum. At first the writer was inclined to the belief that the name *P. penitalis* might apply to the Polygonum species while *P. nelumbialis* could be retained as a valid specific name for the lotus or Nelumbo feeder to which it obviously belongs; but unfortunately Grote described *P. penitalis* from moths reared from larvæ feeding in the seed receptacles of the western water lily (*Nelumbo lutea*). An examination of his types at the American Museum of Natural History in New York leaves no doubt that what he described was not the Polygonum species. The name *P. penitalis*, therefore, must be restricted to the true Nelumbo feeder and *P. nelumbialis* Smith retained as a synonym. Mr. Ainslie succeeded under artificial conditions in rearing *P. ainsliei* to maturity on *Nelumbo lutea*, the food plant of the true *P. penitalis*, but it is doubtful if both species attack that

plant in nature. The natural food plants of *P. ainsliei* are Polygonum, ragweed, and similar plants; and it is frequently found in corn associated with *P. nubilalis*, for which its larva is easily mistaken.

In fact it is impossible to separate the two on superficial characters, and in structure they so closely resemble each other that a careful microscopic examination is necessary to determine which is which. There is a slight difference in the size of the heads of the mature larvæ. That of *P. nubilalis* is slightly larger, as shown by the drawings (Pl. 10, A, G); but this character is comparative and impracticable for purposes of distinction, since it is necessary to have specimens of both species of the same stage of development for comparison and to be certain at the same time of their instars, something that is rarely possible. The shape of the anal plate used by Miss Mosher is unreliable, both species exhibiting the same forms and the same amount of variation. The clear spots indicating certain muscle attachments on the abdomen are scarcely more reliable. The character is extremely elusive and subject to enough modification to leave one in doubt except with most typical specimens. There seems to be only one reliable character—namely, the arrangement of the setæ and puncture of the anterior epicranial group. In *P. nubilalis*, as mentioned, A^2 is approximate to A^1, and A^2, A^1, and the puncture A^a are in a straight line or with the puncture postero-ventrad of A^2. In *P. ainsliei* A^2 is as near to A^3 as to A^1 (or nearer), the three setæ forming almost a right angle with the puncture A^a lying postero-dorsad of A^2, the setæ A^1 and A^2 and the puncture forming an obtuse angle. There is some variation in the degree of distance separating A^1 and A^2 in individual specimens and some asymmetry, especially in *P. nubilalis;* but the character seems to hold, and it has been found sufficiently constant through large series to enable accurate determination of all larvæ so far submitted for identification. The pupa is easily distinguished by the front, which is developed into a knob-like projection (Pl. 9, A). Otherwise it is much like that of *P. nubilalis*, though as a rule smaller and a trifle more slender. The average length is 12 to 14 mm.

PYRAUSTA PENITALIS

Pyrausta penitalis Grote, 1876, *in* Canad. Ent., v. 8, p. 98; Dyar, List No. Amer. Lepidop., p. 391, no. 4439. 1902

Pyrausta nelumbialis Smith, 1890, *in* Ent. Amer., v. 6, p. 89; Dyar, List No. Amer. Lepidop., p. 391, no. 4439. 1902

ADULT

The adult of this species, especially the female, resembles *P. nubilalis* more closely in superficial characters than does *P. ainsliei*. In fresh specimens the darker shadings have a more distinctly ferruginous tint. As in *P. nubilalis*, there is considerable variation among both males and females, and the males have the same sex scaling at the base of the inner margin of the forewing; but the darkest male of *P. penitalis* is never quite so dark as a pale unrubbed specimen of *P. nubilalis*. The hindwing is

pale at the base, and the pale areas bordering the transverse antemedial and transverse postmedial lines of forewing and in the postmedian region of hindwing lack the bright ochreous hue of the male *P. nubilalis*. The yellow is quite as conspicuous in the female, however. Beyond the cell in the forewing of both males and females there is a conspicuous cloudlike blotch of grayish or ferruginous scales, which is much less conspicuous in females of *P. nubilalis* and practically absent in *P. ainsliei*. It is in genitalia characters, however, that the species is most easily and strikingly distinguished. The male genitalia are as figured (Pl. 7, D); apex of tegumen rounded; anellus without anellus lobes but with single, long, stout, ventrally projecting spur (the "calcar" of Pierce)[1]; harpe without spines on inner margin of sacculus; clasper moderate; face of clasper somewhat irregularly oval. Female genitalia (Pl. 8, C, D) without strong chitinization anterior to genital opening; chitinized plates posterior to genital opening pear-shaped, tapering anteriorly. Alar expanse of moths, 20 to 36 mm.

PUPA

The pupa has a very slightly produced front (Pl. 9, B), not a decided knob like that of *P. ainsliei* but more uneven than that of *P. nubilalis*. The dorsal abdominal spines are nearly obsolete and scarcely distinguishable; abdominal spiracles large, rounded, oval; cremaster very stout and characteristic, broader than long (Pl. 9, G); otherwise as in *P. nubilalis*, except that cephalic end is somewhat more sharply tapering and pupa is generally a trifle stouter; average length, 15 to 16 mm.

LARVA

The larva is easily distinguished from that of either *P. nubilalis* or *P. ainsliei*). When full-grown it is 36 to 37 mm. long. The head is considerably larger and the mottling of the head different, the darker pigmentation being in the form of groups of small, distinct spots rather than splotches or continuous masses of blackish brown; the ocellar puncture (O^a) of epicranium lies postero-dorsad of ocellus VI rather than directly posterior to ocellus VI as in *P. nubilalis* and *P. ainsliei;* the mandible (Pl. 11, C) is heavier, oblong rather than square; the median edge is straight or very slightly concaved, not outwardly angulated; and the distal tooth concaved. Except when the parts are greatly distended the shoulder of the stipes maxillaris has a decided hump which is scarcely perceptible in the other two species (Pl. 11, A, B). Crochets of prolegs are rather stout and more evenly triordinal.

DISTINCTIVE CHARACTERS

The three species are very intimately related. In superficial adult characters and in structure of the female genitalia *P. ainsliei* is most readily distinguished. It lacks the sex scaling of the forewing, which is such a prominent character in *P. nubilalis* and *P. penitalis*. On the other hand *P. ainsliei* and *P. nubilalis* are most alike in structure of the male genitalia and hardly separable in larvæ, while *P. penitalis* is readily distinguishable from the other two in both. The adult male of *P. nubilalis* is easily distinguished from all American species of Pyrausta by its dark, smoky, fuscous forewings and hindwings combined with the distinctly yellow color of the lighter areas.

[1] PIERCE, F. N., THE GENITALIA OF THE BRITISH GEOMETRIDAE. 88 p., 48 pl. Liverpool, 1914.

The following tables give the distinguishing structural characters separating the three species:

ADULTS

MALE GENITALIA CHARACTERS

1. Anellus consisting of basal plate (juxta) with single, stout, ventrally projecting spur (calcar) ..... *P. penitalis.*
   Anellus consisting of basal plate (juxta) with two long, slender, dorsally projecting arms (anellus lobes) surrounding aedoeagus ..... 2.
2. Extremity of tegumen rounded ..... *P. ainsliei.*
   Extremity of tegumen trifurcate ..... *P. nubilalis.*

FEMALE GENITALIA CHARACTERS

1. Genital opening strongly chitinized anteriorly ..... *P. ainsliei.*
   Genital opening not strongly chitinized anteriorly ..... 2.
2. Chitinized plates posterior to genital opening decidedly pear-shaped viewed from below ..... *P. penitalis.*
   Chitinized plates posterior to genital opening nearly square viewed from below ..... *P. nubilalis.*

PUPÆ

1. Front evenly rounded or with only slight hump ..... 2.
   Front forming prominent projecting knob ..... *P. ainsliei.*
2. Cremaster broader than long ..... *P. penitalis.*
   Cremaster longer than broad ..... *P. nubilalis.*

LARVÆ

1. Epicranial setæ and puncture A^1, A^2, and A^a lying in a straight line or with A^a somewhat postero-laterad of A^2, not postero-dorsad ..... 2.
   Epicranial setæ and puncture A^1, A^2, and A^a forming an obtuse angle with A^a postero-dorsad of A^2 ..... *P. ainsliei.*
2. Epicranial puncture O^a lying postero-dorsad of ocellus VI; mandible longer than broad; distal tooth concaved ..... *P. penitalis.*
   Epicranial puncture O^a lying directly posterior to ocellus VI; mandible square; distal tooth pointed ..... *P. nubilalis.*

PLATE 7

European corn borer and its nearest American allies:

A.—Male genitalia of *Pyrausta nubilalis;* ventral view of organs spread; aedoeagus omitted.

B.—Male genitalia of *P. nubilalis;* aedoeagus and penis.

C.—Male genitalia of *P. ainsliei;* ventral view of organs spread; aedoeagus omitted.

D.—Male genitalia of *P. penitalis;* ventral view of organs spread; aedoeagus omitted.

E.—Forewing venation of *P. nubilalis;* male.

F.—Hindwing venation of *P. nubilalis;* male.

Explanation of symbols applied to male genitalia.

Ae=aedoeagus.
An=anellus.
Anl=anellus lobes.
Cl=clasper.
Cn=cornutus (thornlike armature of penis).
Cr=calcar.
Hp=harpe.
J=juxta.
Sc=sacculus of harpe.
Ss=subscaphium.
Tg=tegumen.
Ts=transtilla.
Vm=vinculum (sternal portion of ring of the tegumen).

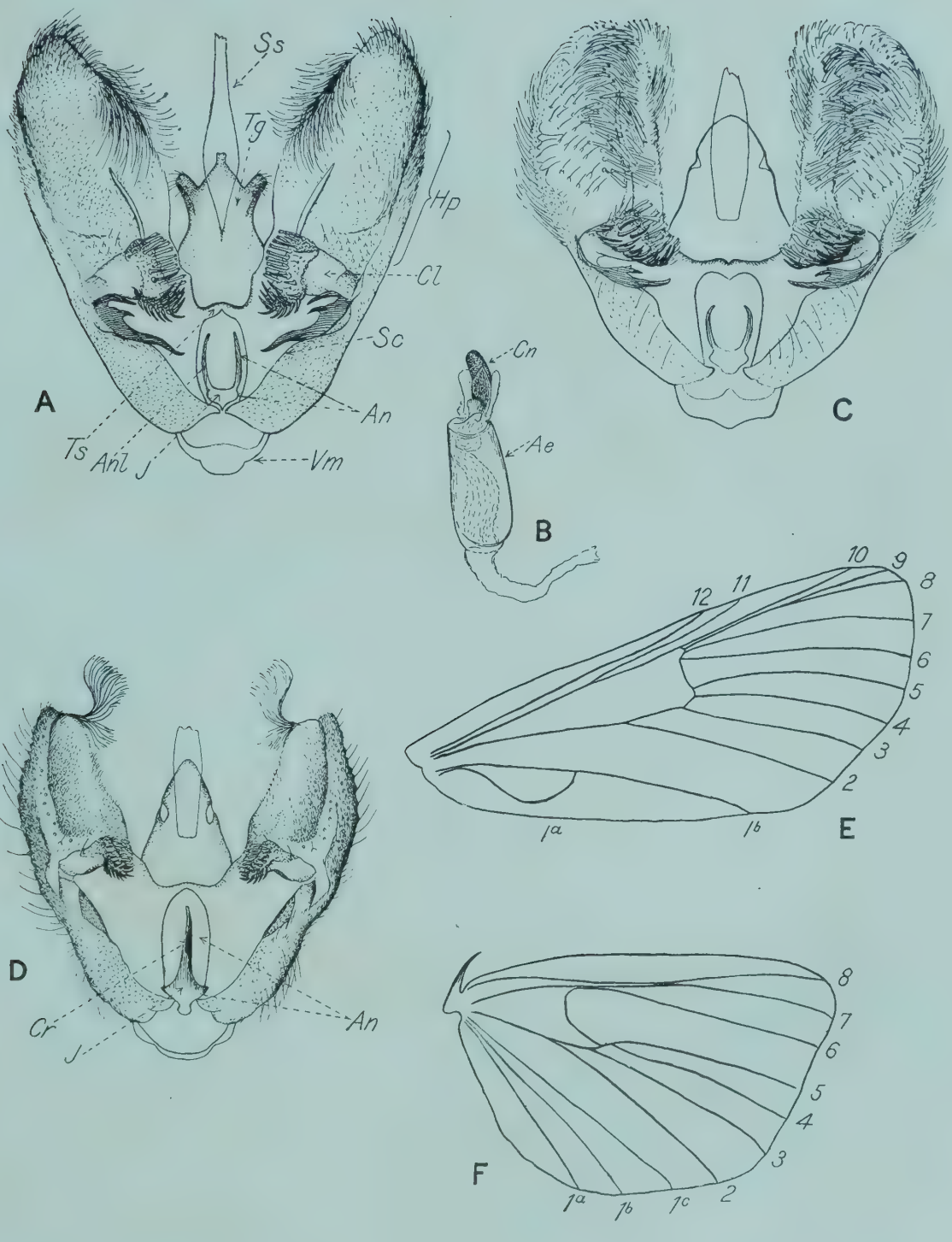
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PLATE 7

European corn borer and its nearest American allies:

A.—Male genitalia of *Pyrausta nubilalis;* ventral view of organs spread; aedoeagus omitted.

B.—Male genitalia of *P. nubilalis;* aedoeagus and penis.

C.—Male genitalia of *P. ainsliei;* ventral view of organs spread; aedoeagus omitted.

D.—Male genitalia of *P. penitalis;* ventral view of organs spread; aedoeagus omitted.

E.—Forewing venation of *P. nubilalis;* male.

F.—Hindwing venation of *P. nubilalis;* male.

Explanation of symbols applied to male genitalia.

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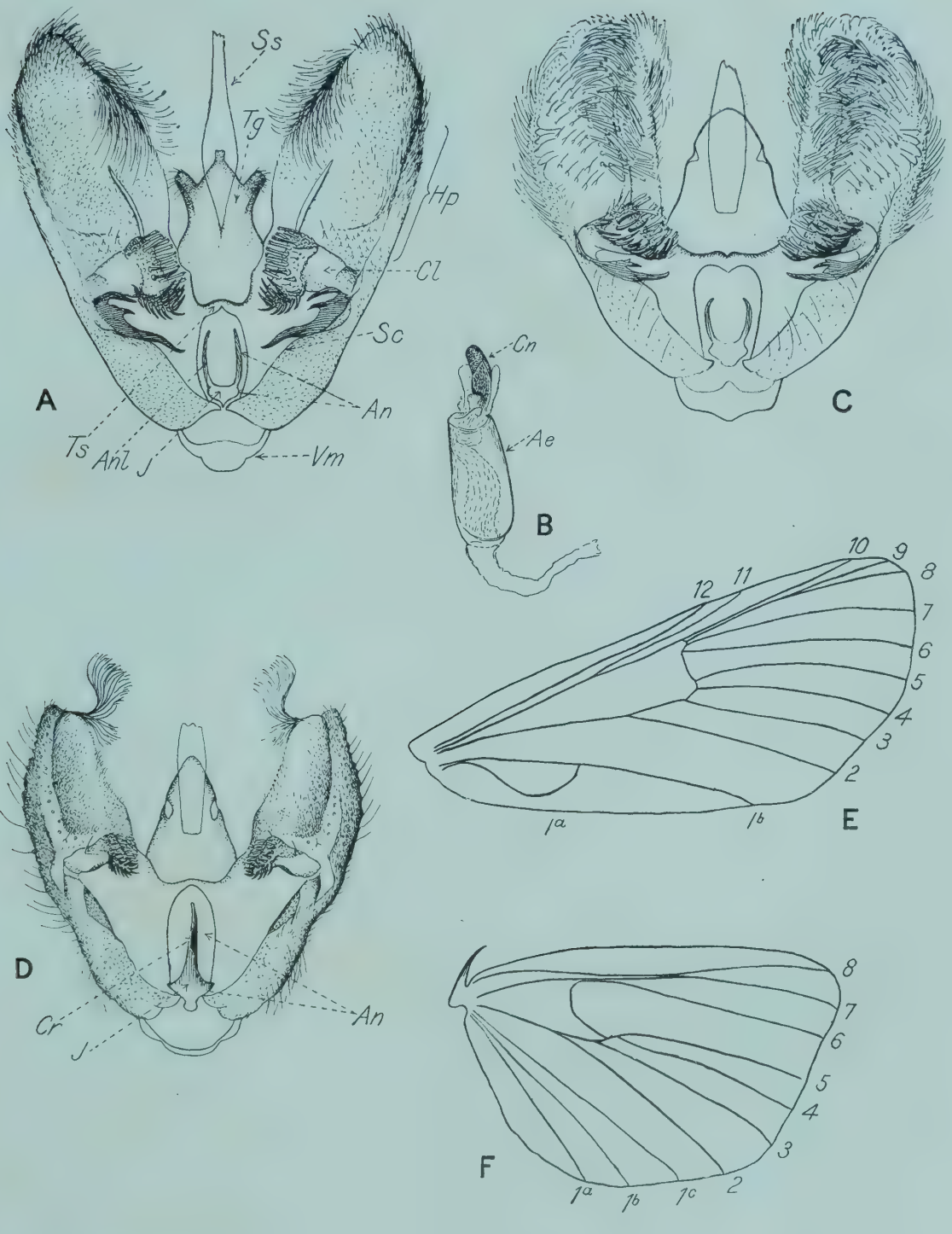
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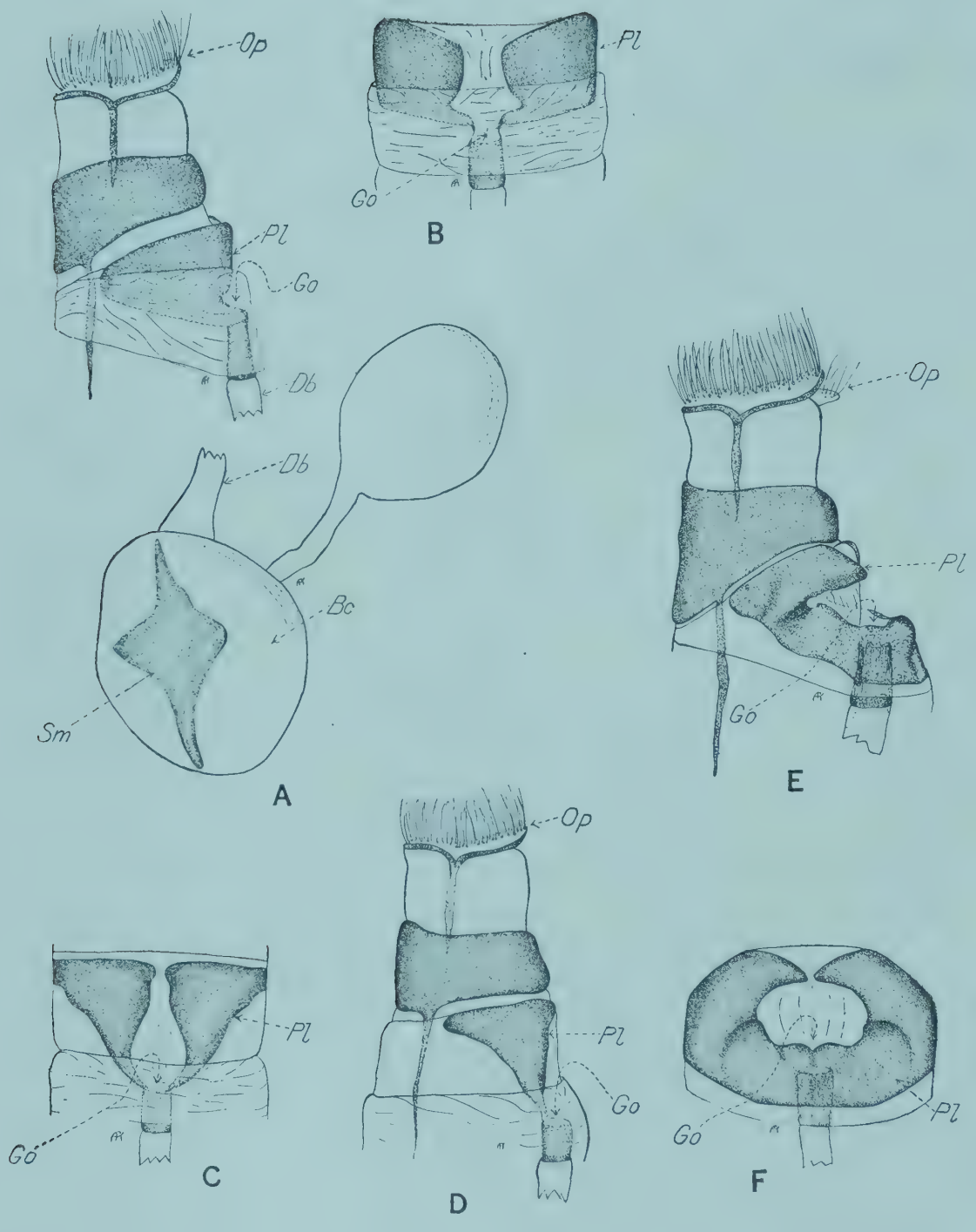
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PLATE 8

Female genitalia of *Pyrausta* spp.:

A.—Female genitalia of *P. nubilalis;* lateral view of organs.

B.—Female genitalia of *P. nubilalis;* ventral view of plates posterior to genital opening.

C.—Female genitalia of *P. penitalis;* ventral view of plates posterior to genital opening.

D.—Female genitalia of *P. penitalis;* lateral view of organs.

E.—Female genitalia of *P. ainsliei;* lateral view of organs.

F.—Female genitalia of *P. ainsliei;* ventral view of plates surrounding genital opening.

Explanation of symbols applied to female genitalia.

Bc=bursa copulatrix.
Db=ductus bursæ.
Go=genital opening.
Op=ovipositor.
Pl=chitinized plates posterior to genital opening.
Sm=signum (internal armature of bursa).

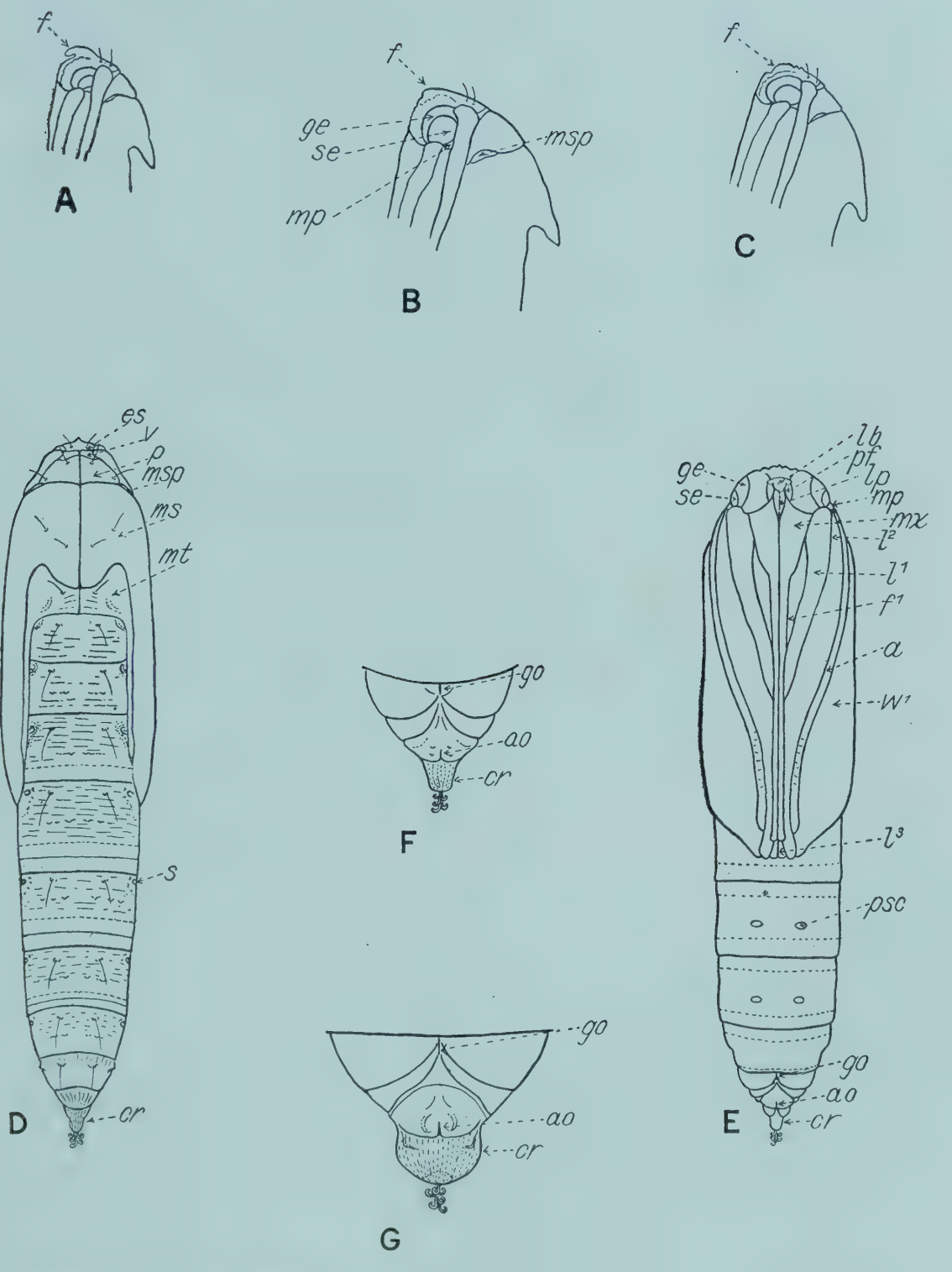
PLATE 9

European corn borer and its nearest American allies:

A.—Profile of cephalic end of pupa of *Pyrausta ainsliei*.
B.—Profile of cephalic end of pupa of *P. penitalis*.
C.—Profile of cephalic end of pupa of *P. nubilalis*.
D.—Pupa of *P. nubilalis*, female; dorsal view.
E.—Pupa of *P. nubilalis*, female; ventral view.
F.—Caudal end of pupa of *P. nubilalis*, female; ventral view.
G.—Caudal end of pupa of *P. penitalis*, female; ventral view.

Explanation of symbols applied to pupæ.

a=antennæ.
ao=anal opening.
cr=cremaster.
es=epicranial suture.
f=front.
f^1=femur of prothoracic leg.
ge=glazed eye.
go=genital opening.
lb=labrum.
l^1=prothoracic leg.
l^2=mesothoracic leg.
l^3=metathoracic leg.
lp=labial palpi.
mp=maxillary palpus.
ms=mesothorax.
msp=mesothoracic spiracle.
mt=metathorax.
mx=maxilla.
p=prothorax.
pf=pilifer.
psc=proleg scar.
s=abdominal spiracle.
se=sculptured eye.
v=vertex.
w^1=mesothoracic wing.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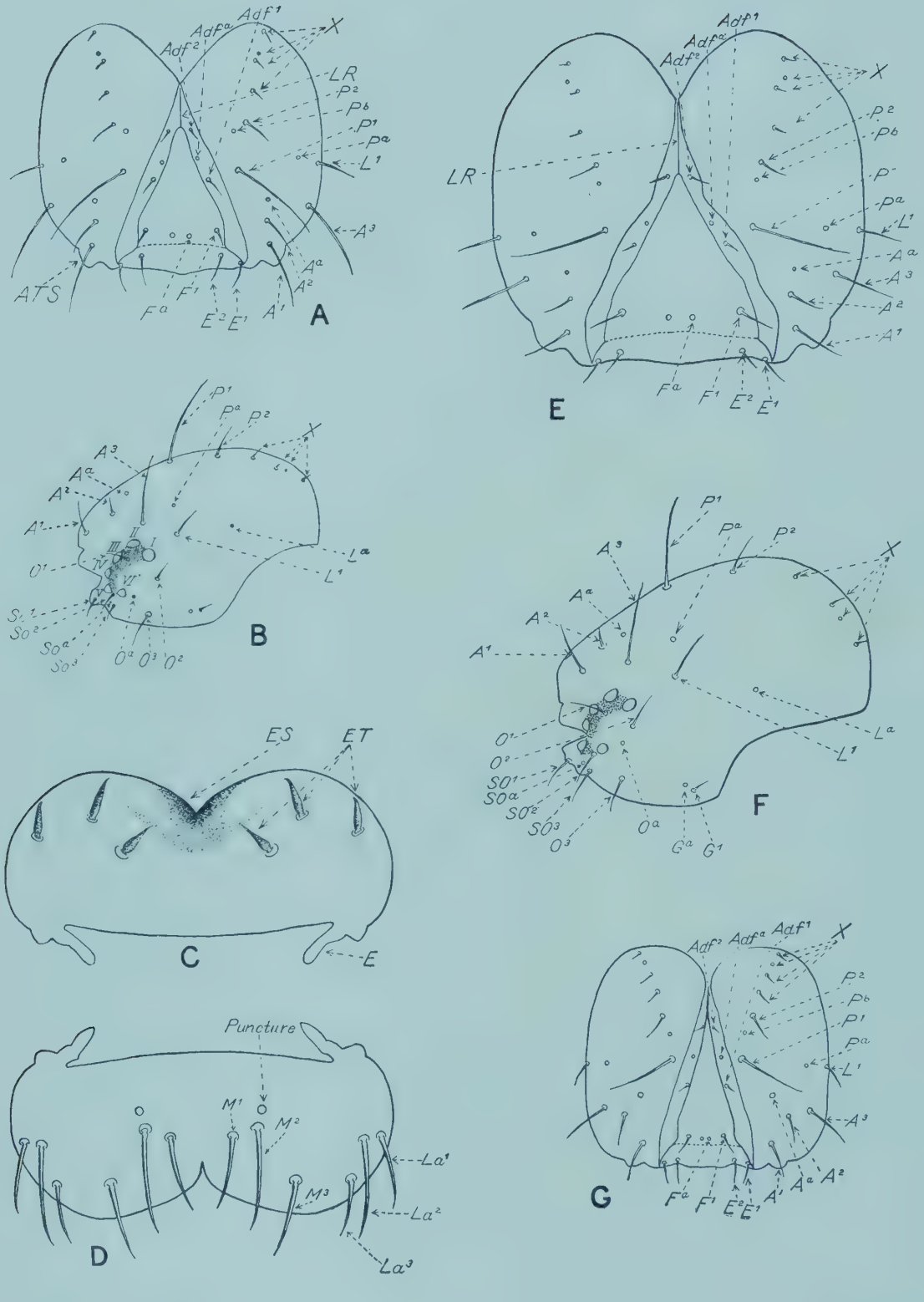
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PLATE 10

European corn borer and its nearest American allies:

A.—Dorsal view of head capsule; larva of *Pyrausta nubilalis*.
B.—Lateral view of head capsule; larva of *P. nubilalis*.
C.—Epipharynx; larva of *P. nubilalis*.
D.—Labrum; larva of *P. nubilalis*.
E.—Dorsal view of head capsule; larva of *P. penitalis*.
F.—Lateral view of head capsule; larva of *P. penitalis*.
G.—Dorsal view of head capsule; larva of *P. ainsliei*.

Explanation of symbols applied to larvæ, Plates 10 and 11.

A^1, A^2, A^3, A^a=setæ and puncture of anterior group of epicranium.
Adf^1, Adf^2, Adf^a=adfrontal setæ and puncture of epicranium.
ATS=antennal shield formed by projection on dorsal surface of epicranium.
C=cardo.
E^1, E^2=epistomal setæ.
ER=epipharyngeal rod.
ES=epipharyngeal shield.
ET=epipharyngeal setæ.
F^1, F^a=frontal seta and puncture.
G^1, G^a=genal seta and puncture.
L^1, L^a=seta and puncture of lateral group of epicranium.
La^1, La^2, La^3=lateral group of setæ of labrum.
LR=longitudinal ridge of epicranium.
M^1, M^2, M^3=median group of setæ of labrum.
mt=thornlike chitinization on proleg bearing abdominal segments.
O^1, O^2, O^3, O^a=setæ and puncture of ocellar group of epicranium.
P^1, P^2, P^a, P^b=setæ and punctures of posterior group of epicranium.
SO^1, SO^2, SO^3, SO^a=setæ and puncture of subocellar group of epicranium.
X=ultraposterior setæ and puncture of epicranium.

PLATE 11

European corn borer and its nearest American allies:

A.—Labium and maxillæ; larva of *Pyrausta nubilalis*.

B.—Left maxilla; larva of *P. penitalis*.

C.—Mandible; larva of *P. penitalis*.

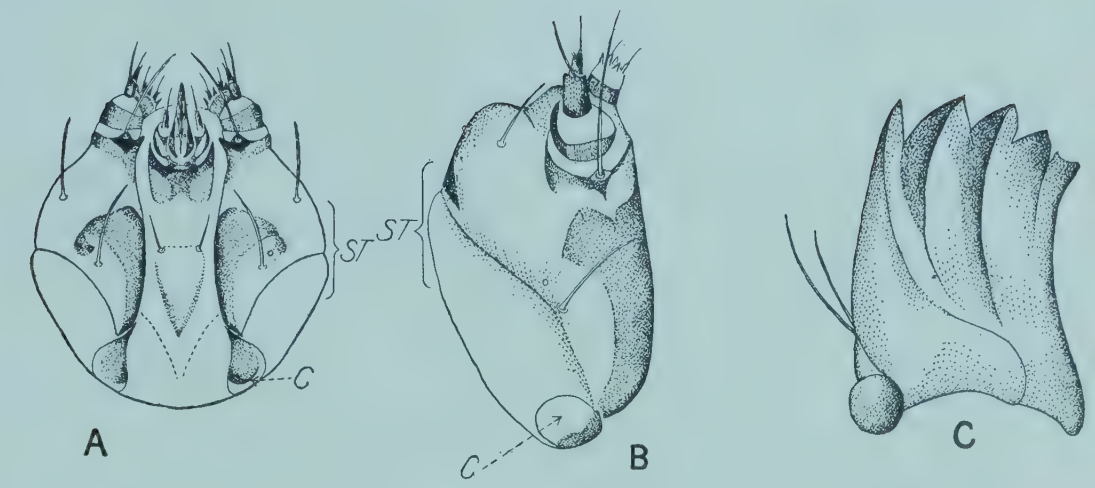
D.—Mandible; larva of *P. nubilalis*.

E.—Character of skin granulations, highly magnified; larva of *P. nubilalis*.

F.—Second thoracic and eighth and ninth abdominal segments of larva of *P. nubilalis*, showing granulose areas above seta VII.

G.—Setal map of prothoracic, mesothoracic, third, eighth, and ninth abdominal segments; larva of *P. nubilalis*.

H.—Crochet arrangement; abdominal proleg; larva of *P. nubilalis*.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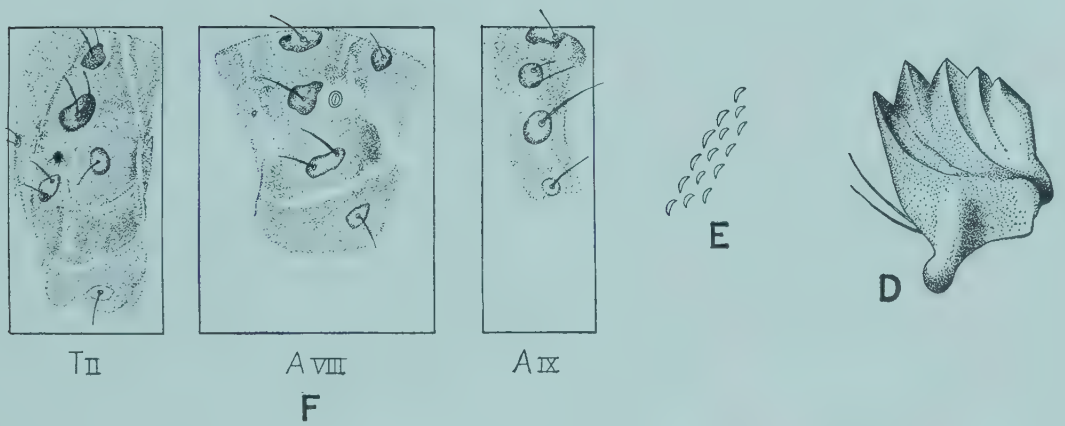
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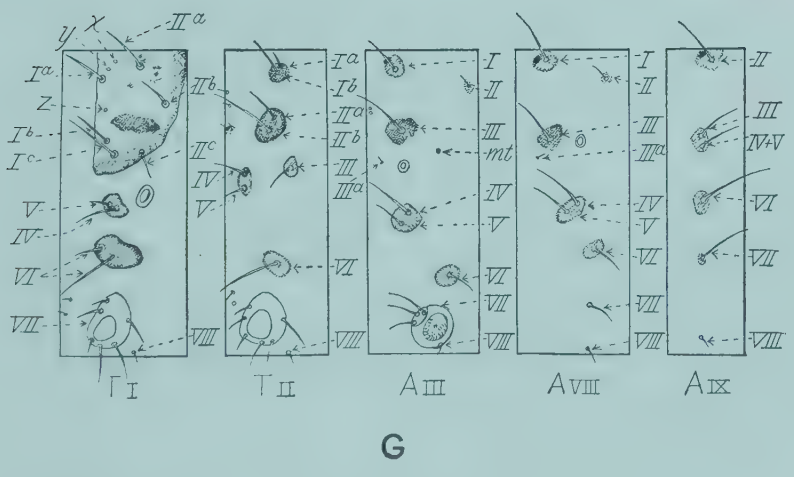
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BACTERIAL BLIGHT OF SOYBEAN

By FLORENCE M. COERPER

Instructor in Plant Pathology, University of Wisconsin [1]

INTRODUCTION

For a number of years a bacterial blight of soybean has been under investigation at the University of Wisconsin. The early observations were made during several seasons prior to 1915 in the university experimental plots by Dr. L. R. Jones and Dr. A. G. Johnson, of the department of plant pathology. In September of that year more intensive study of the trouble was undertaken by the writer. At this time the disease was very severe in Madison fields. Scarcely a plant could be found free from the spotting, and the leaf area of approximately 20 per cent of the crop was destroyed to such an extent as to affect materially the growth of the plants. Subsequent observation has proved the blight to be very prevalent throughout the soybean fields of Wisconsin, and a disease showing symptoms of the same type has been reported from various other localities in the United States.

LITERATURE

Literature, up to this time, gives no detailed description of the disease or of its causal organism, although the malady has, no doubt, been observed in the field for many years. Smith [2] mentions a bacterial leaf-spot of soybean but does not record a thorough study of it. The only other references, with the exception of an abstract [3] published in 1917, seem to be a note by Heald [4] and a later report with figure by Clinton,[5] both of which give short descriptions of the symptoms. There seems no reason to doubt that these workers had under observation the disease described in this paper.

In addition it should be recorded that Manns [6] in 1915 described a bacterial organism pathogenic upon certain legumes, including soybeans.

[1] The writer wishes to make grateful acknowledgements to Dr. L. R. Jones and Dr. A. G. Johnson, of the University of Wisconsin, for supervision and helpful suggestions during the progress of this work.

[2] SMITH, Erwin F. BACTERIA IN RELATION TO PLANT DISEASES. V. 1, p. 92, 1905; V. 2, p. 69, 1911. Washington, D. C. (Carnegie Inst. Washington, Pub. 27.)

[3] JOHNSON, A. G., and COERPER, Florence M. BACTERIAL BLIGHT OF SOYBEAN. (Abstract.) *In* Phytopathology, v. 7, no. 1, p. 65. 1917.

[4] HEALD, Frederick De Forest. REPORT ON THE PLANT DISEASES PREVALENT IN NEBRASKA DURING THE SEASON OF 1905. *In* Nebr. Agr. Exp. Sta. 19th Ann. Rpt., 1905, p. 71. 1906.

[5] CLINTON, G. P. NOTES ON PLANT DISEASES OF CONNECTICUT. *In* Conn. Agr. Exp. Sta. Ann. Rpt., 1915, p. 444–446. 1916.

[6] MANNS, Thomas F. SOME NEW BACTERIAL DISEASES OF LEGUMES AND THE RELATIONSHIP OF THE ORGANISMS CAUSING THE SAME. Del. Agr. Exp. Sta. Bul. 108, 44 p., 21 pl. 1915.

He did not describe nor illustrate the disease on leaves, however; and although the spots on pods and stems, as shown in his plates, do not seem to be characteristically different in type from the lesions caused by the organism discussed in this paper, his report on cultural and morphological studies precludes the possibility of the two organisms being the same.

APPEARANCE OF THE DISEASE

On the leaves, where the disease is very conspicuous, the blight is characterized by small, angular lesions. When young these spots are translucent and water-soaked in appearance and yellow or light brown in color; when old they are dark reddish brown to almost black, very little of the translucency remaining. Single spots are usually from 1 to 2 mm. in diameter and may be quite generally scattered over the leaf surface, although they are often thickly grouped and confluent, resulting in irregular lesions several millimeters in diameter or even so large as to involve considerable portions of the leaf. Frequently, also, the spots seem to take the course of the principal leaf veins, and marginal infections are not unusual. Very often a yellowing of the tissue surrounding the lesion occurs. In advanced stages the invaded tissues may become dry and fall out, thereby giving the leaf a very ragged appearance (Pl. A).

Evidence all points strongly to the fact that the bacterial blight of soybean is not confined to leaves alone but that it affects also the stems, petioles, and pods. Black lesions on the stems and petioles, varying in size from very small spots to those of considerable length and breadth, are often found accompanying the trouble on the leaves, and water-soaked spots from which drops of exudate were oozing have also been found on young petioles (Pl. 12, A).

On the pods the disease appears first as small water-soaked spots, often showing exudate droplets (Pl. 12, B, C). The lesions may grow much larger, sometimes involving a considerable part of the pod. They usually turn to dark brown or black with age, the exudate drops frequently drying down as brownish nubs or scales. The seeds within diseased pods also become affected and may be found covered with a slimy bacterial growth (Pl. 13, A, B).

Isolations made from petioles and pods such as are here described have produced typical lesions when applied to soybean leaves, and re-isolations have yielded the typical organism.

Under the lens or even with the unaided eye, glistening exudate may often be observed in small quantities on the underside of the leaf spots. This ooze seldom appears as droplets in the field, except under very favorable moisture conditions, but infected tissue allowed to remain in a damp chamber for a number of hours is often covered with tiny drops of the grayish white exudate. These, however, on exposure to the air, dry very readily as inconspicuous brownish granules or scales, or seem to disappear entirely.

SEASONAL OCCURRENCE

The seasonal development of the bacterial blight has been under observation in a number of soybean fields in Wisconsin during the past four years, and a variety of conditions has been found to exist. Sometimes the disease will appear with the first leaves and progress steadily throughout the season. In other cases, the first leaves may show infection, then there may be a considerable amount of healthy growth, and later a further development of the disease on newer, younger tissue may occur. Sometimes, also, there has been no apparent development of the lesions until late July or early August, when the spotting might appear in abundance and continue until the plants were mature.

It seems reasonable to believe that weather conditions, especially those of moisture, have a great influence in determining the nature of the development and progress of the disease. However, since in the vicinity of Madison, Wis., all the above conditions have been observed in different fields during the same season, other factors than weather must have some importance in influencing the amount of disease present in any given locality. Just what these other factors may be is still an open question, since up to this time not enough experimental evidence has been accumulated to warrant making definite statements. The amount of seed infection suggests itself at once as having some relation, as do also the possibility of the persistence of the organism in the soil and the degree of exposure of the soybean plants to the blight organism. Moreover, field observations have shown that a real difference in varietal resistance exists. A further discussion of these relations will be given under the subject of control.

THE ORGANISM

ISOLATION

Microscopic observations of sections of invaded tissue show the interior of the lesions to be swarming with bacteria. The usual method of isolating the organism has been by the use of poured agar plates.

In the orignal isolation a portion of freshly invaded tissue was cut out, washed thoroughly through 10 changes of sterile water, and macerated upon a slide under as sterile conditions as possible. This material was introduced into sterile bouillon, further dilutions made, and plates poured, Thaxter's potato hard agar being the medium employed. In three days both yellow and white colonies had appeared on the plates. Characteristic colonies, both yellow and white, were picked and transfers made to potato agar slants. Water suspension inoculations with subcultures from several of these strains proved a white organism to be the pathogene. This conclusion was verified by subsequent re-isolation and re-inoculation. In no case did a yellow strain cause infection.

In later work the organism has been obtained with almost complete suppression of the yellow species, which are, no doubt, surface organisms, by dipping a piece of diseased tissue in 95 per cent alcohol for an instant, immersing in mercuric chlorid (1 to 1,000) for one minute, and then rinsing through five or six sterile water blanks before macerating and plating out.

When exudation has been abundant and the tissue clean, successful isolations have been also made by touching an exudate drop with a sterile platinum needle and transferring directly to an agar slant. This method has proved especially satisfactory when the exudate has been forced out in a damp chamber from leaves artificially infected in the greenhouse, although isolations have been made from field material in the same way.

FIG. 1.—*Bacterium glycineum:* From 72-hour growth on potato agar, stained by Duckwall's method to show flagella. × 2,000.

In the investigation of this disease a number of strains of the causal organism have been used in inoculation as well as in cultural studies. One of these, designated A, which has proved especially virulent in both greenhouse and field experiments, has been studied intensively and is presented as the type strain. This isolation was made from a leaf lesion in 1917. Some of the other isolations will be considered in a comparative way later in this paper.

MORPHOLOGY

The organism is a medium-sized rod with rounded ends and occurs usually singly or in pairs. When stained from 2-day-old potato agar cultures with Ziehl's carbol fuchsin or gentian violet, the cells average about 2.3μ long by 1.2μ wide. When stained with Duckwall's flagellum stain the average measurement is about 3μ by 1.5μ.

Both Zettnow's and Duckwall's flagellum stains have shown the organism to be motile by means of one to several polar flagella (fig. 1). No endospores or involution forms have been observed. Capsules were not demonstrated when stains were made from potato or beef-peptone agar cultures, but well-developed capsules were found present on blood agar. Both Welch's and Hiss's methods of staining were used. Light flocculent and sometimes membranous pellicles developed on the surface of certain liquid media such as bouillons of favorable reactions, sugar solutions, and on Fermi's and Uchinsky's solutions. The organism is gram negative.

CULTURAL CHARACTERS

Unless otherwise specified, all cultures were incubated at 25° C., a temperature very favorable for the growth of the organism. Reactions were determined by titration with phenolphthalein as indicator, the trial solution always having been boiled previous to test. All references to reaction of media are recorded in terms of Fuller's scale. Ridgway's color standards [1] were used in the determination of color.

AGAR POURED PLATES.—On potato agar, colonies appeared in about 48 hours and at the end of 5 days were from 2 to 5 mm. in diameter. They were creamy white tinged with brown, circular, shining, and convex with umbonate center. The margin in general was entire, although it may be slightly lobed. The surface may show indication of irregular wrinkling or beading in the central part, or there may be more or less concentric and radiating convolutions throughout the colony with a definite growth border.[2] The consistency was butyrous. Buried colonies were lenticular.

On +10 beef-peptone agar, colonies appeared in about 48 hours and at the end of 5 days were about 2 to 3 mm. in diameter. They were circular, smooth, shining, and convex. The margin was entire, with no noticeable surface irregularity. The colonies were creamy white in color tinged with brown, butyrous in consistency, and showed a definite browning of the medium around them. This brown color (chestnut) later became general throughout the entire plate. Buried colonies were lenticular.

AGAR STABS.—Stabs in potato agar when 2 days old showed a surface growth about 6 mm. in diameter, rather flat, shining, and creamy white tinged with brown. Later this growth may spread over three-fourths to almost the entire surface. Definite but moderate growth followed the stab. There was no change in the medium.

Stabs in +10 beef-peptone agar when 3 days old showed a surface growth about 5 mm. in diameter, rather flat, shining, creamy white tinged with brown, with slight browning of the medium to the depth of the stab. Later the top growth may become more spreading, involving a considerable part of the surface. Definite growth, a little more marked than on potato agar, followed the stab. The medium finally became quite uniformly browned.

AGAR SLANTS.—On potato agar slants, stroke cultures made a moderate, shining, flat, filiform to irregularly scalloped growth, creamy white in color tinged with brown. More or less wrinkling may occur on the surface. At low temperatures the growth was thicker and more

[1] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C., 1912.

[2] The irregularities are not always clearly evident to the naked eye. They show best under magnification and when lighting is semidirect. There is also considerable variation in the degree of surface marking of different strains of the blight organism (Pl. 15).

piled up, more shining and less wrinkled. The consistency was butyrous. Old cultures became dull, and the brownish tinge deepened.

On +10 beef-peptone agar, growth was slower and less abundant than on potato agar. It was also thinner, and of finer consistency. Surface irregularities were usually present as was the unevenly scalloped margin, though these characteristics were not always conspicuous. The color was creamy white tinged with brown. In about three days the agar began to take the chestnut-brown color observed in the plate and stab cultures. It was darker directly under the streak, and in a week the medium usually was uniformly browned. The agar discolored more slowly when cultures were grown at low temperatures or under adverse conditions.

GELATIN PLATES.—At room temperature (about 21° C.), the colonies were usually small on +10 peptone gelatin plates. There was no liquefaction of the medium, but it soon turned chestnut-brown in color.

GELATIN STABS.—At room temperature in +10 peptone gelatin, the surface growth was spreading after several days. There was no liquefaction, but the medium became a deep chestnut-brown about one-third of the distance down from the top. There was slight indication of growth following the stab.

POTATO CYLINDERS.—Growth on steamed potato cylinders in two days was spreading, rather flat, shining, slimy, and more or less viscid in consistency, smooth, without odor, and yellowish white in color. The growth turned the potato brownish gray.

MILK.—Inoculated milk coagulated slowly. In a month there was a fine curd developed, but no decided separation. The cultures took on a cream color and had a sort of half-transparent appearance. In two months a thin, somewhat clear layer developed on top, with the half-transparent-looking curd below.

LITMUS MILK.—Lavender-colored litmus milk cultures began to turn blue at the top three days after inoculation. In six days they were quite uniformly blue and remained without further change for about a month, when separation occurred. In seven weeks there had developed a thin, darker blue, rather clear layer on top, with fine, soft curd below and a small amount of cream-colored precipitate at the bottom.

METHYLENE BLUE IN MILK.—At the end of two days whitening began at the bottom and proceeded gradually upward. In about two weeks the cultures had become fairly white throughout with a blue layer at the top only. After this the blue color returned slowly, followed by a second whitening process which in about six weeks from the time of inoculation was complete, except for a thin layer at the top of the cultures. Curd began to form about this time, giving the cultures a half-transparent appearance. In two months there was a thin, somewhat clear, greenish blue layer on top with semitransparent, creamy, soft curd below. There was no marked separation.

NITRATE BOUILLON.—Nitrate bouillon cultures in fermentation tubes gave good clouding in the open arm but none in the closed arm. No gas was produced. In all cases there was a definite line of demarcation across the U of the tube. There was a fair positive test for ammonia and a strong positive test for nitrates. No nitrites were found. The medium in the open arm became so dark brown in color that it was impossible to test with any degree of accuracy for acidity.

FERMENTATION TUBES.—The tests were made in 2 per cent Difco peptone water and 2 per cent of each of the following carbon compounds: Dextrose, lactose, saccharose, maltose, glycerin, and mannit. Clouding was first observed at the end of 24 hours. In a week good growth had developed in all tubes in the open arm. With all of the sugars except dextrose, considerable cloudiness developed also in the closed arm, but no gas was found in any tube. Tests proved that there was growth in the closed arm of the dextrose culture tubes also; but here it was more or less slimy, and clear rather than cloudy. *Bacterium coli* was used as the control in this test, and gas was produced in all *Bact. coli* tubes.

When the cultures were 2 weeks old they were titrated, and they were tested again at the end of five weeks. Phenolphthalein was used as the indicator, and in every test there was preliminary boiling to drive off the carbon dioxid. At the end of two weeks the maltose, lactose, glycerin, and mannit cultures showed considerable increase in alkalinity. When 5 weeks old, however, the tests showed that acid was being produced again, and in some instances the cultures were more acid than the controls. The maltose and lactose cultures had turned so dark brown in the open arm that it was difficult to determine the exact endpoint in the titrations, but several trials made from each tube where the medium was not so dark-colored gave a satisfactory check on the results.

After two weeks' growth the dextrose and saccharose cultures showed, in general, increased acidity. In a number of instances, however, the tests made from the closed arms indicated an increase in alkalinity there. At the end of the 5-week period all the cultures showed an increase in acid in both open and closed arms.

The condition just described, which at first glance looks a bit confusing, may be explained by the following theory: The organism grows very rapidly in the dextrose and saccharose solutions where it at first causes an alkaline reaction, followed by the production of acid. The change does not take place so rapidly in the closed arm, and for this reason titration after two weeks still showed increased alkalinity. The open arm had gone beyond this point, and acid was being produced there in excess. Gradually, as we have seen, the acid condition became general throughout the tubes. No doubt the same action took place in the lactose, maltose, glycerin, and mannit cultures already described, except that there the changes occurred more slowly.

Nestler's reagent was used for the ammonia test. The maltose and lactose cultures gave a fair positive reaction; the other carbohydrates gave a very weak test for ammonia.

TITRATION OF SODIUM CHLORID.—Tubes of neutral, peptonized beef bouillon, containing, respectively, 0.5, 1, 1.5, 2, 3, and 4 per cent chemically pure sodium chlorid, were inoculated from 5-day-old potato agar cultures. In about a week there was fair clouding in the 0.5 per cent and the 1 per cent strengths. No growth occurred in any other tubes.

OPTIMUM REACTION AND TOLERATION LIMITS.—Peptonized beef bouillon was used for this test. Hydrochloric acid was the acid employed and sodium hydrate was the alkali. Bouillons were prepared, titrating +25, +22, +20, +18, +15, +10, +5, 0, −5, −10, −20, and −25, and were inoculated as uniformly as possible from 6-day-old potato agar cultures. At the end of 48 hours growth was visible in +18, +15, +10, +5, and 0; and in a week growth was very good in these cultures, with a light flocculent pellicle. Browning, working down from the top, began in these from the seventh to the ninth day, and the color continued to deepen to a reddish brown (chestnut) throughout. The +10 and +15 cultures were darker in color than the +5, and these in turn were darker than those at 0 and +18. The +10 culture showed heaviest growth. In a month +18, +15, +10, and +5 cultures showed no more clouding; but there was a precipitate at the bottom, especially heavy in the +10 tubes. This precipitate broke up readily on shaking. The 0 cultures at this time were still cloudy, but there was also a precipitate which broke up easily on shaking.

Growth developed slowly in the −5 reaction, browning also taking place more slowly than with the other cultures. A light, flocculent pellicle developed in some cultures. In a month these tubes showed various shades of brown, but they were no longer cloudy. There was a precipitate which broke up on shaking.

Slow growth took place in two tubes of +20, which also turned chestnut color in about a month, though they still remained slightly cloudy. There was a heavy precipitate which was easily broken up on agitating.

None of the other reactions showed growth.

Apparently, therefore, +10 Fuller's scale seems to represent the optimum reaction

FERMI'S SOLUTION.—Tubes of Fermi's solution inoculated from young potato agar cultures developed good clouding in 4 days. A flocculent pellicle began to form in about a week and was heavy in 14 days. Slight fluorescence appeared in about 2 weeks and later became fairly decided. The pellicle began to settle in about 5 weeks; and finally there was a heavy, flaky, and somewhat stringy precipitate at the bottom of the cultures.

USCHINSKY'S SOLUTION.—Inoculation was made from young potato agar cultures. Clouding was apparent in 4 days and was heavy in 6

days, with a light, fluocculent pellicle forming, which later became heavy. Fluorescence appeared in about 10 days and persisted throughout the entire test, 7 weeks. At the end of this time the pellicle had disappeared; but there was a heavy, flaky precipitate at the bottom of the cultures.

COHN'S SOLUTION.—The organism grew slowly in Cohn's solution. No fluorescence appeared.

STARCH AGAR.—There was no evidence of diastatic action on potato starch suspended in beef-peptone agar, when tested with potassium iodid-iodin.

INDOL.—Erlich's test for indol was used with +10 beef-peptone bouillon as the medium. There was a weak, positive reaction from the third to the seventh day. After this time the cultures had grown too dark to give the color test.

BLOOD SERUM.—Stroke cultures on solidified blood serum gave a moderate, rather flat growth, smooth, shining, and creamy white tinged with brown. The medium was not liquefied.

AEROBISM.—The organism behaved as a weak facultative anaerobe. Clouding occurred in the closed arm of the fermentation tubes with all the sugars tested except dextrose. Definite though not vigorous growth occurred below the surface in stab cultures. Stroke cultures of agar also gave indication of slight growth for some distance below the surface.

LITMUS AGAR WITH SUGARS.—Litmus-lactose and litmus-maltose stroke cultures developed good growth. In six days the medium began to brown under the streak and finally turned a rich reddish brown throughout. This is the same sort of change that takes place in all beef-peptone media. There was no apparent reddening or bluing due to the predominating production of acid or alkali, but, judging from the test with carbohydrates in fermentation tubes, it is probable that such changes took place and were masked by the browning of the medium.

On litmus-dextrose agar there was abundant growth and a distinct acid reaction. In seven days the cultures had become decidedly scarlet. In about six weeks the red color disappeared again, leaving the cultures about the same color as the controls.

TEMPERATURE RELATIONS

Cultures on potato agar and in +10 beef-peptone bouillon proved the optimum temperature to be between 24° and 26° C. No growth occurred at 35° either in bouillon or on agar. Rather slow but fairly heavy growth occurred both on agar and in bouillon at 2°. The extreme minimum was not determined.

Several trials have shown the thermal death point of 2-hour cultures made from 2-day-old cultures to be 48° to 49° C. for freshly isolated, vigorous strains. Consistent results have been secured in trials of three different years. Isolations which have been cultured for some time,

however, show that there is a gradual lowering of the death point with age. In a recent test (1919) it was found that cultures from a 1915 strain were practically all killed at 46° to 47°, whereas trials with this same strain in 1916 had shown the normal death point to be 48° to 49°. In the 1919 trials all tubes of a 1917 isolation developed fair growth after being subjected to 47°, but there was no growth after 48°. Cultures of a 1918 strain showed good growth in a number of the tubes after 48°, although clouding was not uniform throughout. In no test, however, has there been any clouding after 49° or above.

DESICCATION

The organism seems not to be extremely resistant to drying as it occurs in the host tissue. Isolations attempted from herbarium material 1 and 2 years old failed to yield the organism. The vitality varies on different culture media. On potato agar, where growth is very abundant, the organism usually dies within two months, whereas on beef-peptone agar and in bouillon it lives considerably longer. Transfers made from 6-months-old cultures of these last-named media developed good growth. Also cultures kept at a low temperature in a moist atmosphere, such as that afforded by an ice box, seemed to retain their vitality for a much longer time than those allowed to remain at a higher temperature in a dry incubator.

When dried on sterile cover glasses the organism was found to live only a comparatively short time. In these tests a young, vigorously growing bouillon culture was diluted with an equal amount of sterile water and a 2-mm. loop of this dilution transferred to sterile cover glasses which were then dried in a sterile chamber. When these cover glasses were dropped at intervals into tubes of sterile bouillon, it was found that the organism was not alive after 65 hours of drying.

TECHNICAL DESCRIPTION

Bacterium glycineum n. sp.[1]

Cylindrical rods, rounded at ends, solitary or in pairs; individual rod 2.3 to 3μ by 1.2 to 1.5μ; motile by 1 to several polar flagella; aerobic to weakly anaerobic; no spores; capsulated when grown on blood agar.

Superficial colonies on potato agar plates round, shining, convex with umbonate center and surface irregularities; creamy white tinged with brown; margin slightly lobed.

No liquefaction of gelatin; does not digest casein; nitrates not reduced; cultures in various carbohydrate media produce acid with no gas. Gram negative.

Group number, 222.2223032.

Pathogenic on *Glycine hispida* Maxim., forming angular lesions which are seriously destructive to leaves. Affects also pods and other aerial parts.

Type locality: Madison, Wis., on *Glycine hispida* Maxim.

Distribution: Widespread.

[1] According to Migula's classification, the combination would be *Pseudomonas glycineum*, n. sp.

VARIATIONS AMONG STRAINS

As has already been suggested, a number of other strains of the blight organism have been isolated and studied in comparison with strain A, and during the progress of this work certain interesting variations have been found to exist.

The original isolation, strain E, was obtained from a leaf lesion in the fall of 1915, was studied in the greenhouse and culturally in the laboratory during 1916, and has been used in comparison with the type strain, A, in all inoculation and culture work since that time. Its original pathogenicity was proved; but its virulence, which was never so great as that of A, seems to be practically lost after three years.

In culture, strains A and E behave very much alike, but with one marked difference. It has been shown that strain A causes a consistent browning of certain media, such as beef-peptone broth, beef-peptone agar, and peptone solution plus certain sugars; whereas strain E does not cause this color change. Also, strain E, under favorable condition for growth, develops, on potato agar, colonies and streak cultures showing a decidedly wrinkled and convoluted surface. On the other hand, while colonies and slope cultures of strain A, grown on potato agar, may develop a more or less irregular surface also, ordinarily they appear much smoother than those of E and do not make such an abundant growth. There is in addition a slight color difference, strain A showing on potato agar a browning tinge which is absent in E until the cultures are rather old.

A number of isolations made during the seasons of 1917 and 1918 from leaf, petiole, and seed lesions correspond with strain A in color and in the ability to brown peptone media. In general, colonies and slope cultures of these isolations, on potato agar, compare more favorably with strain E in surface irregularity, although there are smooth types among them. These later strains have not been studied in detail, but they have all proved to be pathogenic on soybean leaves and are apparently the same as the type leaf strain. The writer also has in reserve certain isolations made from stem and leaf which correspond with the original strain E in grosser cultural characters and in the apparent inability to cause browning of the peptone media. It is hoped that the pathogenicity of these strains may be determined during the coming season. One strain of this type, recently isolated from a leaf which developed natural infection in the greenhouse, has produced typical lesions on soybean seedlings.

On beef-peptone agar no structural differences between colonies and streaks of different strains appear. Here we find simply the difference in ability to brown the medium. Plates 14 and 15 show the lack of uniformity in the surface characteristics of colonies and slope cultures of pure, authentic strains of the soybean blight organism. It will be

noted that considerable variation may occur among colonies on the same plate.

Just what the significance of these variations may be is rather difficult to state without more thorough study of this part of the cultural work. We have found, however, that both types of the organism are pathogenic on soybean leaves and are able to produce typical lesions. Furthermore, there have been no marked differences in morphology or in cultural behavior. It seems, therefore, the justifiable disposition of the matter, at least for the present, to consider that we are dealing with forms of a slightly variable species. Since these forms agree in the characters ordinarily included in descriptions of bacterial species as well as in pathogenicity and host reactions, it does not seem wise nor helpful to segregate them formally by technical description or varietal name. Should subsequent investigations indicate more adequate bases or need for this segregation it may then be made.

INOCULATION EXPERIMENTS

During the years 1916 to 1918, the bacterial leaf spot has been reproduced many times and in typical form under greenhouse and field conditions by artificial inoculation (Pl. 16, 17). From lesions produced in this way, the original type organism has been repeatedly recovered. In the early work the tissues were wounded at the time of inoculation, but it was soon found that simply spraying water suspensions of the blight organism upon uninjured leaves was sufficient to produce infection, not only when the tissue was very young and tender but also when the leaves had more nearly approached maturity. It has been found advisable, however, in most cases, since soybean leaves are very hairy and therefore prevent liquid from spreading readily upon them, to rub the inoculated leaves gently between thumb and finger in order to insure contact between the inoculum and the leaf surface.

In making greenhouse inoculations the following method was usually employed: Soybeans were sown in 8-inch pots in sterile soil, and when a few leaves had developed they were inoculated with an atomizer spray of a water suspension of the organism. The plants were then allowed to remain in a damp chamber for from 48 to 72 hours, after which they were removed to a greenhouse bench.

In making inoculations in the field, practically the same method was employed. Plants perfectly free from the disease were selected, the leaves inoculated with atomizer spray and then covered with glasine paper bags which were removed after two or three days. In field work the younger leaves of almost mature plants were most often used in the inoculation tests.

Control plants were always sprayed with sterile water and otherwise treated in the same manner as inoculated plants. Both inside and out

of doors typical lesions usually began to develop in about 10 days after the inoculum was applied.

Only one attempt was made to inoculate pods. This experiment was performed in the field in September, 1917, when young pods were simply sprayed with a water suspension of strain A. Typical lesions developed in almost every pod, whereas the controls remained absolutely free from spots (Pl. 13, B).

RELATIONS TO HOST TISSUE

Razor sections of lesions in fresh leaves show the bacterial invasion to be in the parenchymatous tissue. Critical histological studies have not been made, but leaf lesions fixed in Gilson's fixative, embedded in paraffin, sectioned, and stained in carbol fuchsin have shown intercellular invasion. Infection evidently takes place through the stomata.

OVERWINTERING, DISSEMINATION, AND CONTROL

Practically no experimental work has been done up to this time concerning the bacterial blight organism in relation to overwintering and dissemination. It has been noted, however, that the disease appears year after year in the same field and also that it may appear almost as soon as the first leaves have developed, although it must be remembered that this latter condition is not at all universal and that the lesions sometimes make their first appearance when the plants are nearing maturity. These facts seem to indicate that the organism may be carried with the seed and also that it may live over in the soil. Pods and seeds often become badly affected with the disease as we have already seen and, although we have learned that the organism does not withstand long periods of desiccation, at least in the leaves, there is a possibility that it may live a much longer time upon the seed, or, judging from the nature of the invasion, within it.

Until further work on overwintering and dissemination has been done it will be impossible to recommend any specific control measures aiming to check the development and spread of the disease in the field. Destroying all diseased plants, rotation, and the selection of sound seed for planting have been suggested as possible means of control; but it will be seen at once that the first recommendation is impracticable from the standpoint of soybean culture; and, considering how generally the disease occurs, we can not entertain very great hopes of the other two in practice. Considerable observational data have been accumulated, however, during the last few years concerning varietal resistance and susceptibility to the soybean blight, and there seems to be no question that decided differences in varietal resistance exist. During the last few seasons the writer has observed repeatedly, in variety test plots, perfectly healthy plants growing immediately adjacent to others

almost completely destroyed by the bacterial blight. In fact, very often the leaves of the two plants were in actual contact and yet this difference in susceptibility to the disease persisted throughout the season. Inoculation experiments in the greenhouse have strengthened the belief that certain varieties of soybeans are immune to the attacks of the blight organism (Pl. 18), and organized experimental work is now under way at the University of Wisconsin on this phase of the problem. It seems very probable from evidence at hand that the bacterial blight might at present be greatly mitigated if not satisfactorily controlled by the intelligent selection of disease-resistant varieties from among those already in use. If it appears that the ideal combination of disease resistance with other desirable characters does not occur in the standard varieties now preferred, it should be possible to combine these by properly directed breeding efforts.

SUMMARY

(1) The blight of soybean described in this paper has been observed in the University of Wisconsin plots for several years. A disease, undoubtedly the same, has been reported from various parts of the United States.

(2) It is characterized on the leaves, where it is most conspicuous, by small, angular spots, either isolated or confluent. The lesions are light-colored and translucent in early stages and very dark-colored in late stages. In late stages, also, the diseased tissue may become dry and drop out, giving the leaves a ragged appearance. Bacterial exudate occurs on the leaf spots as droplets but is not very evident except under favorable moisture conditions. It is pale in color and dries as inconspicuous granules or scales.

(3) Petiole, stem, and pod lesions accompany the disease on the leaves. Exudation has been observed on petioles and pods.

(4) The blight is caused by *Bacterium glycineum*, n. sp., which is able to make entrance into the tissues without wounds. The organism is a medium-sized rod, motile by means of from one to several polar flagella. In culture its color is creamy white tinged with brown.

(5) Isolation of the organism has been accomplished repeatedly by macerating a thoroughly washed lesion upon a slide under sterile conditions and using this material for dilutions from which agar plates were poured. Cultures made by transferring from characteristic colonies or from fresh exudate droplets to agar slants have furnished the material used in the inoculation work.

(6) Simply spraying water suspensions of the organism upon soybean plants is sufficient to produce infection. It is advisable, however, to rub the tissue gently between thumb and finger to insure contact between inoculum and plant surface.

(7) Best growth of the organism occurs between 24° and 26° C. The maximum is about 35°. The absolute minimum has not been determined. Slow growth develops at 2°.

(8) The organism is sensitive to desiccation, and there seems to be gradual loss in pathogenicity when it is grown in artificial culture.

(9) Certain variations in cultural behavior exist between different strains of the soybean blight organism, including the ability of some strains—for example, the type—and inability of others to brown the peptone media. Studies to date have led us to consider, at least for the present, that we have been dealing with forms of a slightly variable species.

(10) Invasion occurs in the parenchyma.

(11) Control measures are not yet fully worked out. At present there seems to be greatest promise in the development of disease-resistant varieties.

PLATE A

Bacterial spot on leaves of soybean.[1]

[1] This plate was prepared under the direction of Dr. M. W. Gardner, Bureau of Plant Industry, United States Department of Agriculture.

A Hoen & Co Baltimore

PLATE 12

Bacterium glycineum:

A.—Soybean petiole. Natural infection, showing exudate droplets. × 6.

B.—Blighted soybean pod. Natural infection, showing young water-soaked lesions. × 6.

C.—Blighted soybean pod. Natural infection, showing exudate droplets. × 6.

B
A
C

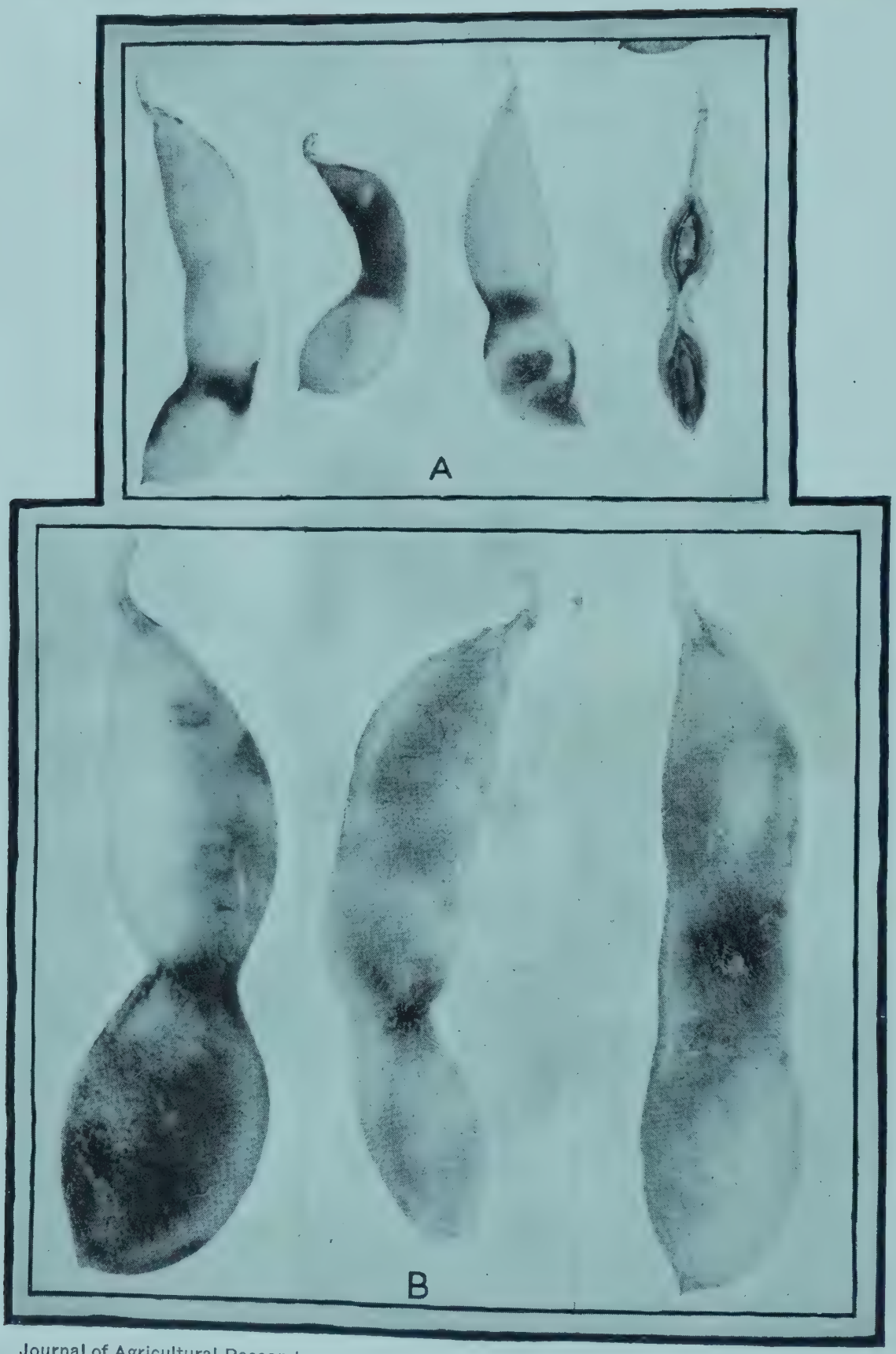
A
B

PLATE 13

Bacterium glycineum:

A.—Natural infection on soybean pods. Note also seed infection in pod at right. × 1.

B.—Artificial infection on soybean pods inoculated with type strain. × 2.

PLATE 14

Bacterium glycineum:

A.—Six-day-old potato agar slant culture, strain E.

B and C.—Six-day-old potato agar slant cultures, type strain. Note difference in degree of surface marking.

A
B
C

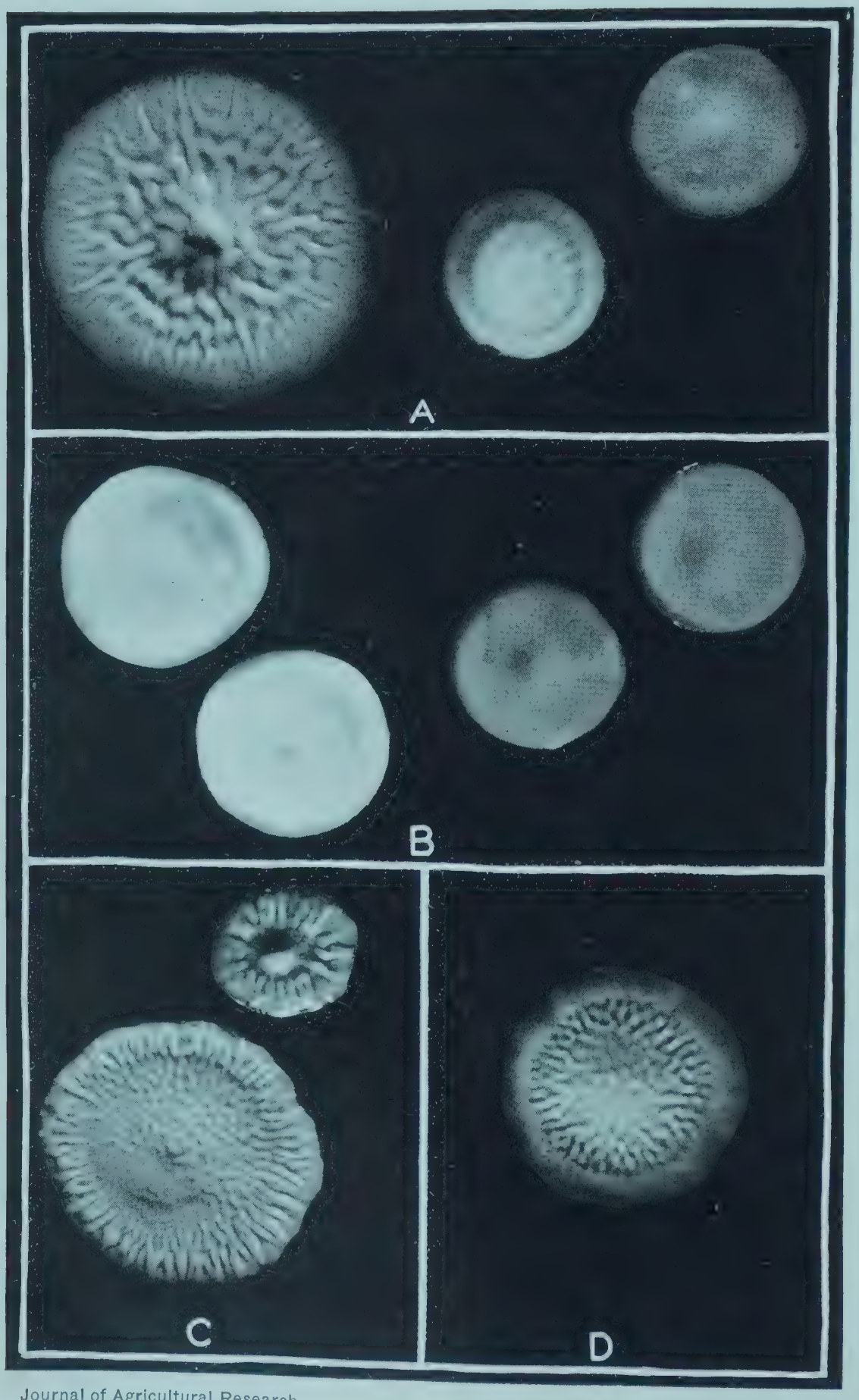
A
B
C
D

PLATE 15

Bacterium glycineum:

A.—Colonies of petiole strain on potato agar plate.

B.—Colonies of type strain on potato agar plate. Surface irregularities present but not conspicuous.

C.—Colonies of strain E on potato agar plate.

D.—Colony of seed strain on potato agar plate. All plates were dilution cultures from bouillon. Note the differences in surface marking even among colonies on the same plate.

PLATE 16

Bacterium glycineum:

Soybean seedlings showing infection after inoculation in the greenhouse with type strain. Control plant at right.

PLATE 17

Bacterium glycineum:

Blighted soybean leaves artificially inoculated in the field with type strain. The two leaves at the top of the page were punctured previous to use of atomizer spray; the center leaf was simply sprayed; the two lower leaves were sprayed and then rubbed lightly.

PLATE 18

Bacterium glycineum:

Soybean seedlings artificially inoculated in the greenhouse with type strain. Two plants at left, susceptible variety. Plant at right, resistant.

DOMOLD SPORES CONTAIN ENZYMS?

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Molds have long been known to produce intracellular and extracellular enzyms, but so far as we have been able to ascertain no study has been made of the enzyms contained in the spores of these organisms. This is indeed noteworthy, since the valuable contributions of Duclaux, Fernbach, and others mentioned by Effront[2] and Dox[3] have apparently been concerned solely with the enzymic activities of molds in the mycelial stage. It has been shown by us in another connection[4] that the deterioration of sugar occurred where mold mycelia developed and in certain instances where spores alone were present. This phenomenon gave rise to the query, Do mold spores contain enzyms? This is the concern of the present investigation. It is our purpose to limit the scope of this article to the invertase activity of the spores of *Aspergillus niger* (*Aspergillus sydowi* [Bain. and S.]), *Aspergillus flavus*, and *Penicillium expansum*, because of the economic significance of this enzym and the wide distribution of these molds.

METHOD OF PROCEDURE

A large number of Petri dishes containing Kopeloff's agar[4] were seeded with a single pure, bacteria-free culture of the desired mold and incubated at 35° C. for six days. A small amount of sterile distilled water was introduced into each plate, shaken slightly, and then poured on sterilized filter paper (Whatman No. 4). The mycelium was then washed through the filter paper with sterile distilled water, and the spores were separated by a combination of filtration and flotation. This process was continued long enough to accumulate the desired quantity of spores. The spores were then rinsed off the filter paper into a sterile Erlenmeyer flask of 300-cc. capacity. To 250 cc. of this spore suspension and an equal volume of sterile distilled water, 1 cc. of c. p. chloroform was added.

[1] The authors are indebted to Director W. R. Dodson, Assistant Director W. G. Taggart, Dr. F. W. Zerban, and Mr. E. C. Freeland for their kind interest and assistance, and to Mr. W. L. Owen for reading the manuscript.

[2] EFFRONT, Jean. ENZYMES AND THEIR APPLICATIONS . . . English translation by Samuel C. Prescott. 322 p. New York, 1902.

[3] DOX, A. W. THE INTRACELLULAR ENZYMES OF PENICILLIUM AND ASPERGILLUS . . . U. S. Dept. Agr. Bur. Anim. Indus. Bul. 120, 70 p. 1910.

[4] KOPELOFF, Nicholas, and KOPELOFF, Lillian. THE DETERIORATION OF CANE SUGAR BY FUNGI. La. Agr. Exp. Sta. Bul. 166.

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An examination of the number of spores present per cubic centimeter was made by means of the blood-counting cell, as described by us in another connection.[1] Unfortunately it was impossible to disintegrate the clumps, and the reported counts are consequently approximate. In this instance there were about 2,000,000 spores per cubic centimeter of inoculum. The flasks were then heated to 63° C. for 30 minutes for the purpose of killing the spores[2] and making the spore wall more permeable. The contents of each flask were then transferred to Erlenmeyer flasks containing sterile washed sand and shaken vigorously for 5 minutes in order to cause further rupture in the spore walls. The inoculum was then ready to be added to the 200-cc. portions of 10 per cent (by weight) granulated sugar solution in cotton-plugged Erlenmeyer flasks, which had been previously sterilized in the autoclave at 15 pounds pressure for 15 minutes. All the flasks had the same polarization. Ten cc. of sterile distilled water were added to the control flasks, and 10 cc. and 20 cc. of inoculum were added to the others. After this inoculation both the inoculum and sterile distilled water were heated to 100° for 20 minutes to kill any enzym which might be present. Then this sterilized inoculum was added to the sugar solution as explained above. All flasks were then incubated at 35° to 45° for 3 hours. At the end of this time 50 cc. of solution were removed with a sterile pipette, filtered, and polarized. Reducing sugars were determined in the same sample by the modified Violette method (volumetric). The results are recorded in Table I. The actual polarization values and the average percentage of decrease in sucrose are given in separate columns.[3]

It will be seen from the results in Table I that the flasks inoculated with sterile distilled water and those inoculated with sterilized inoculum, heated to 100° C., had the same polarization (35.7), within experimental error, and the same amount of reducing sugars (0.04 per cent), while the flasks having 10 cc. of spores heated to 63° polarized 34.8, which represents a loss in polarization of 0.9 as compared with the control, or a decrease of 0.23 per cent sucrose. The reducing sugars increased 0.02 per cent. The inoculum of spores was polarized alone and exhibited no optical activity. However, where 20 cc. of spores heated to 63° were used, the polarization dropped to 34.1, which was a decrease of 1.5 as compared with the control, or an actual decrease of 0.38 per cent sucrose. The reducing sugars were slightly increased. Where 20 cc. of inoculum were used it is necessary to correct for the added dilution, if we wish to compare these results with those obtained with 10 cc. of inoculum.

[1] KOPELOFF, Nicholas, and KOPELOFF, Lillian. OP. CIT.

[2] THOM, Charles, and AYERS, S. Henry. EFFECT OF PASTEURIZATION ON MOLD SPORES. *In* Jour. Agr. Research, v. 6, no. 4, p. 153–166, 3 fig. 1916.

[3] The authors wish to thank Mr. E. C. Freeland, Assistant Chemist, for his kind assistance with these analyses.

Furthermore, it must be remembered that throughout these results each polarization value represents the optical activity of all three sugars present—sucrose, dextrose, and levulose—and consequently can not be accepted unreservedly as an adequate criterion of inversion; rather we must consider the reducing sugars for such a purpose. From these results it is evident that after three hours the enzymic activity of the spores of *Aspergillus niger* was already manifest. A microscopic examination of the contents of each flask was made for the purpose of detecting any germination or bacterial contamination, if present. No germination or contamination occurred. Thus the enzymic activity, as evidenced by the reduction in polarization and increase in reducing sugars, was produced by the spores of the mold present. That this inversion was truly enzymic is proved by the fact that when the spores were heated to 100°, which temperature kills all enzym activity, the inoculation induced no change in the composition of the original sugar solution.

TABLE I.—*Analyses of 10 per cent sugar solutions inoculated with spores of Aspergillus niger*

[Three hours' incubation]

Flask No.	Treatment.	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
				Per ct.	*Per ct.*	*Per ct.*
3	10 cc. sterile water heated to 63° C.	35.6	.....	.....	0.03	.....
4	.....do.....	35.6	.....	.....	.04	.....
	Average.....	35.6	.....	.....	.04	.....
12	10 cc. sterile water heated to 100° C.	35.8	.....	.....	.04	.....
14	.....do.....	35.8	.....	.....	.04	.....
	Average.....	35.8	.....	.....	.04	.....
5	10 cc. spores heated to 100° C.	35.8	.....	.....	.04	.....
6	.....do.....	35.6	.....	.....	.04	.....
	Average.....	35.7	.....	.....	.04	.....
15	10 cc. spores heated to 63° C.	34.9	.....	.....	.05	.....
16	.....do.....	34.8	.....	.....	.06	.....
17	.....do.....	34.8	.....	.....	.07	.....
	Average.....	34.8	0.9	0.23	.06	0.02
18	20 cc. spores heated to 63° C.	32.0	.....	.....	.07	.....
19	.....do.....	32.3	.....	.....	.06	.....
20	.....do.....	32.1	.....	.....	.07	.....
	Average.....	32.1	3.6	.90	.07	.03
	Corrected to 10 cc.....	34.1	1.5	.38	.07	.03

TABLE II.—*Analyses of 10 per cent sugar solutions inoculated with spores of Aspergillus niger*

[Twenty-four hours' incubation]

Flask No.	Treatment.	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
				Per ct.	*Per ct.*	*Per ct.*
3	10 cc. sterile water heated to 63° C.	35.6	.....	.....	0.05	.....
4	.....do.....	35.6	.....	.....	.05	.....
	Average.....	35.6	.....	.....	.05	.....
12	10 cc. sterile water heated to 100° C.	35.8	.....	.....	.....	.....
14	.....do.....	36.0	.....	.....	.04	.....
	Average.....	35.9	.....	.....	.04	.....
5	10 cc. spores heated to 100° C.	35.8	.....	.....	.04	.....
6	.....do.....	35.8	.....	.....	.04	.....
	Average.....	35.8	.....	.....	.04	.....
15	10 cc. spores heated to 63° C.	31.1	.....	.....	.20	.....
16	.....do.....	31.0	.....	.....	.20	.....
17	.....do.....	31.0	.....	.....	.20	.....
	Average.....	31.0	4.6	1.16	.20	0.15
18	20 cc. spores heated to 63° C.	26.1	.....	.....	.44	.....
19	.....do.....	25.9	.....	.....	.50	.....
20	.....do.....	26.0	.....	.....	.50	.....
	Average.....	26.0	9.6	2.41	.48	.43
	Corrected to 10 cc.....	27.2	8.4	2.11	.50	.45

The flasks each received three drops of chloroform and were returned to the incubator at 45° C., where they remained until analyzed at the end of the 24-hour incubation period. They were frequently shaken to assist in the rupturing of the spore walls. From the results recorded in Table II it will be noted that, as in Table I, the flasks inoculated with sterile water heated to 63° and 100° as well as the sterilized inoculum—spores heated to 100°—polarized about 35.8 and contained about 0.04 per cent reducing sugars. On the other hand, there was a marked decrease in polarization in the flasks inoculated with 10 cc. of spores heated to 63°. This is shown by the value 31, which represents a total loss of 4.6 or a decrease of 1.16 per cent sucrose. The reducing sugars increased appreciably to 0.15 per cent from 0.05 per cent. Still more striking is the fact that double this quantity of spores (20 cc.) was responsible for a proportional decrease in polarization—amounting to 8.4, or a decrease of 2.11 as compared with the control—and a slightly greater increase in reducing sugars. Again a microscopic examination disclosed no signs of

germination or contamination. Thus the results after 24 hours confirmed those obtained after 3 hours' incubation, and we must conclude that the spores of *Aspergillus niger* had released sufficient of the enzym invertase to carry forward the inversion of sucrose already noted.

Finally, after the addition of three drops of chloroform and another incubation, the flasks were analyzed at the end of four days. The results are to be found in Table III.

TABLE III.—*Analyses of 10 per cent sugar solutions inoculated with spores of Aspergillus niger*

[Four days' incubation]

Flask No.	Treatment.	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
				Per ct.	*Per ct.*	*Per ct.*
3	10 cc. sterile water heated to 63° C.	37.3	.....	.....	0.05	.....
4	.....do.....	37.2	.....	.....	.05	.....
	Average.....	37.3	.....	.....	.05	.....
12	10 cc. sterile water heated to 100° C.	36.8	.....	.....	.04	.....
14	.....do.....	36.8	.....	.....	.04	.....
	Average.....	36.8	0.5	0.12	.04	.....
5	10 cc. spores heated to 100° C.	37.0	.....	.....	.04	.....
6	.....do.....	36.8	.....	.....	.04	.....
	Average.....	36.9	.4	.09	.04	.....
15	10 cc. spores heated to 63° C.	23.9	.....	.....	.98	.....
16	.....do.....	24.7	.....	.....	.95	.....
17	.....do.....	23.7	.....	.....	.87	.....
	Average.....	24.1	13.2	3.31	.93	0.88
18	20 cc. spores heated to 63° C.	18.2	.....	.....	1.84	.....
19	.....do.....	17.9	.....	.....	1.90	.....
20	.....do.....	18.2	.....	.....	2.27	.....
	Average.....	18.1	19.2	4.80	2.00	1.95
	Corrected to 10 cc.....	18.9	18.4	4.55	2.09	2.04

Compared with the previous analyses the control flasks—sterile water heated to 63° and 100° C. and spores heated to 100°—show a slightly higher and somewhat less consistent polarization. This is undoubtedly due to evaporation, the flasks having been plugged with cotton. That the discrepancies are negligible is evidenced by the uniformity in amount of reducing sugars present, which represent the true criterion of inversion. Where 10 cc. of spores heated to 63° were employed, the polarization was reduced to 24.1 as compared with 37.3 in the control, a loss of 13.2 or a decrease of 3.31 per cent sucrose. The reducing sugars indicate

a correspondingly high increase of 0.88 per cent. Where 20 cc. of inoculum were used it will be observed that the polarization was reduced 18.4 as compared with the control, or a decrease of 4.55 per cent sucrose, and the reducing sugars were increased to 2.04 per cent. Again no germination or contamination could be detected in a microscopic examination.

Since the particular problem which gave rise to the present investigation—namely, the deterioration of sugar—is concerned primarily with solutions of high concentration, it was planned to corroborate the above evidence by repeating the experiment and using a sugar solution of 20 per cent by weight, or double the concentration of that previously employed. The methods and technic were identical with those described in the experiment just reported, except that in this case there were fewer spores present in the inoculum.

TABLE IV.—*Analyses of 20 per cent sugar solutions inoculated with spores of Aspergillus niger*

[Twenty-four hours' incubation]

Treatment.	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 cc. sterile water heated to 63° C.	61.0	.....	.....	0.05	.....
10 cc. sterile water heated to 100° C.	61.2	.....	.....	.05	.....
10 cc. spores heated to 100° C.....	62.2	.....	.....	.05	.....
10 cc. spores heated to 63° C.....	58.0	3.0	0.72	.38	0.33
20 cc. spores heated to 63° C.....	52.9	8.1	1.94	1.21	1.16

In Table IV are recorded the results of the analyses after 24 hours' incubation at 45° C. Only the averages of closely agreeing triplicate determinations are here presented, and corrections for amount of inoculum are calculated. It will be observed that the polarization and reducing sugars of the flasks receiving 10 cc. of sterile distilled water heated to 63° and those heated to 100° were the same Ten cc. of spores heated to 100° gave a slightly higher polarization, but these variations are probably the results of the sterilization process, which did not affect all flasks in an identical manner. However, there is no question concerning the reduction in polarization as effected by 10 cc. of spores heated to 63°, which amounts to 3, or a decrease of 0.72 per cent sucrose. The reducing sugars show a somewhat greater increase—0.33 per cent. Where 20 cc. of spores were employed, the polarization was 52.9 as compared with 61 in the control flasks, a reduction of 8.1 or a decrease of 1.94 per cent sucrose. Correspondingly there was an increase in reducing sugars of 1.16 per cent. No germination or contamination could be detected by a microscopic examination. Thus the results obtained with a 20 per cent sugar solution confirmed those previously obtained

with the 10 per cent solution and form an adequate basis for establishing the fact that the spores of *Aspergillus niger* contain invertase.

TABLE V.—*Summary of enzymic activity of spores of Aspergillus niger*

Quantity of spores.	Concentration of sugar solution.	Incubation period.	Decrease in polarization.	Decrease in sucrose.	Decrease in sucrose.[a]	Increase in reducing sugars.	Increase in reducing sugars.[a]
Cc.	*Per cent.*	*Hours.*		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
10.....	10	3	0.9	0.23	2.5	0.02	50
10.....	10	24	4.6	1.16	13.0	.15	300
10.....	20	24	3.0	.72	4.9	.33	660
10.....	10	96	13.2	3.31	35.4	.88	1,760
20[b].....	10	3	1.5	.38	4.2	.03	75
20[b].....	10	24	8.4	2.11	21.9	.45	900
20[b].....	20	24	8.1	1.94	13.3	1.16	2,320
20[b].....	10	96	18.4	4.55	50.0	2.04	4,080

[a] Original considered as 100 per cent. [b] Corrected to 10 cc.

In Table V the data previously presented are summarized in such a way as to make evident the correlations which are of interest. For example, it will be readily observed that with a given quantity of inoculum in a sugar solution of definite concentration there is a progressive decrease in polarization accompanied by an increase in reducing sugars with an increase in the incubation period. Again, it may be noted that increasing the inoculum causes practically a proportional decrease in polarization and a corresponding increase in reducing sugars. This lends further support to the evidence brought forward that spores contain enzyms, for it is precisely under such circumstances that a relationship is established between the number of spores and the amount of enzymic activity. While the data presented do not establish such a correlation with mathematical accuracy, one may assume that the number of spores whose walls become ruptured would be somewhat variable in quantity, and consequently a progressive increase in enzymic activity may be seen to accompany an increase in the number of spores.

It is especially interesting to note that 20 cc. of spores reduced the polarization of a 10 per cent sugar solution in 4 days in such a way as to indicate the loss of 50 per cent of the original sucrose present. This inoculum accounted for the loss of 22 per cent of the original sucrose content in 24 hours. The increase in reducing sugars is even more striking, as seen in the last column of Table V, where, in the cases mentioned above, the amounts were increased 4,080 and 900 per cent, respectively, compared with the original percentage taken as 100.

When the inoculation of a 10 per cent sugar solution with 10 cc. of spores is compared with a similar inoculation of a 20 per cent sugar solution, it will be seen that in 24 hours the amount of reducing sugars in the latter case was about double that in the less concentrated solution.

A similar phenomenon is to be observed when 20 cc. of spores are employed. This is in line with the theoretical considerations of the activity of the enzym invertase and indicates that the amount of inversion depends upon the quantity of sucrose present. The polarization values do not permit of such clearly defined generalization because of the more complex nature of the factors involved.

The procedure described above was repeated, using the spores of *Penicillium expansum* as an inoculum; and the results are recorded in Table VI. The calculations for diminished and increased dilution depending upon the amount of inoculum added have been incorporated.

TABLE VI.—*Analyses of 10 and 20 per cent sugar solutions inoculated with spores of Penicillium expansum*

10 PER CENT SUGAR SOLUTION

Treatment.	After three hours' incubation.				
	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 cc. sterile water heated to 73° C.	38.5	.....	.....	0.04	.....
10 cc. sterile water heated to 100° C.	38.5	.....	.....	.04	.....
10 cc. spores heated to 100° C.	38.5	.....	.....	.04	.....
5 cc. spores heated to 63° C.	38.5	.....	.....	.04	.....
10 cc. spores heated to 63° C.	38.5	.....	.....	.04	.....
20 cc. spores heated to 63° C.	38.4	0.1	0.02	.06	0.02

20 PER CENT SUGAR SOLUTION

10 cc. sterile water heated to 63° C.	73.2	.....	.....	0.04	.....
10 cc. sterile water heated to 100° C.	73.2	.....	.....	.04	.....
10 cc. spores heated to 100° C.	73.2	.....	.....	.04	.....
5 cc. spores heated to 63° C.	73.2	.....	.....	.06	0.02
10 cc. spores heated to 63° C.	73.0	0.2	0.05	.15	11
20 cc. spores heated to 63° C.	72.6	.6	.15	.23	.19

It will be readily observed that with 5 and 10 cc. of inoculum no inversion took place in a 10 per cent sugar solution, and only a very slight increase in reducing sugars is to be found where 20 cc. were used. The inoculum contained 600,000 spores per cubic centimeter and exhibited no optical activity. However, where a 20 per cent solution was employed the reducing sugars increased with an increase in inoculum, while there was a substantial decrease in polarization and percentage of sucrose with the largest inoculum.

The increase in inversion which occurred with an increase in incubation period is not large and is in agreement with the facts which were noted after three hours' incubation. Thus it may be inferred that the spores of *Penicillium expansum* have a relatively slight invertase content.

The analyses at the end of 24 hours and 3 days, respectively, are presented in Tables VII and VIII.

TABLE VII.—*Analyses of 10 and 20 per cent sugar solutions inoculated with spores of Penicillium expansum*

10 PER CENT SUGAR SOLUTION

Treatment.	After 24 hours' incubation.				
	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 cc. sterile water heated to 63° C.	38.5	.....	.....	0.04	.....
10 cc. sterile water heated to 100° C.	38.5	.....	.....	.04	.....
10 cc. spores heated to 100° C.	38.5	.....	.....	.04	.....
5 cc. spores heated to 63° C.	38.5	.....	.....	.04	.....
10 cc. spores heated to 63° C.	38.5	.....	.....	.04	.....
20 cc. spores heated to 63° C.	38.4	0.1	0.02	.05	0.01

20 PER CENT SUGAR SOLUTION

10 cc. sterile water heated to 63° C.	73.4	.....	.....	0.04	.....
10 cc. sterile water heated to 100° C.	73.4	.....	.....	.04	.....
10 cc. spores heated to 100° C.	73.5	.....	.....	.04	.....
5 cc. spores heated to 63° C.	72.7	0.7	0.17	.08	0.04
10 cc. spores heated to 63° C.	73.1	.3	.07	.18	.14
20 cc. spores heated to 63° C.	72.6	.8	.19	.29	.25

TABLE VIII.—*Analyses of 10 and 20 per cent sugar solutions inoculated with spores of Penicillium expansum*

10 PER CENT SUGAR SOLUTION

Treatment.	After three days' incubation.				
	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 cc. sterile water heated to 63° C.	39.5	.....	.....	0.04	.....
10 cc. sterile water heated to 100° C.	39.5	.....	.....	.04	.....
10 cc. spores heated to 100° C.	39.5	.....	.....	.04	.....
5 cc. spores heated to 63° C.	39.1	0.4	0.10	.05	0.01
10 cc. spores heated to 63° C.	38.3	1.2	.30	.06	.02
20 cc. spores heated to 63° C.	38.1	1.4	.35	.05	.01

20 PER CENT SUGAR SOLUTION

10 cc. sterile water heated to 63° C.	74.4	.....	.....	0.04	.....
10 cc. sterile water heated to 100° C.	74.4	.....	.....	.04	.....
10 cc. spores heated to 100° C.	74.4	.....	.....	.04	.....
5 cc. spores heated to 63° C.	73.6	0.8	0.19	.14	0.10
10 cc. spores heated to 63° C.	72.6	1.8	.44	.18	.14
20 cc. spores heated to 63° C.	72.7	1.7	.42	.32	.28

The same procedure was presently followed, using the spores of *Aspergillus flavus* as an inoculum to the extent of 300,000 per cubic centimeter. The results are to be found in Table IX.

TABLE IX.—*Analyses of 10 and 20 per cent sugar solutions inoculated with spores of Aspergillus flavus*

10 PER CENT SUGAR SOLUTION

Treatment.	After three hours' incubation.				
	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 cc. sterile water heated to 63° C.	39.5	.....	.....	0.04	.....
10 cc. sterile water heated to 100° C.	39.5	.....	.....	.04	.....
10 cc. spores heated to 100° C.	39.5	.....	.....	.04	.....
10 cc. spores heated to 63° C.	39.5	.....	.....	.08	0.04
10 cc. spores heated to 63° C.	39.5	.....	.....	.14	.10
10 cc. spores heated to 63° C.	40.1	.....	.....	.07	.07

20 PER CENT SUGAR SOLUTION

10 cc. sterile water heated to 63° C.	72.0	.....	.....	0.04	.....
10 cc. sterile water heated to 100° C.	72.0	.....	.....	.04	.....
10 cc. spores heated to 100° C.	72.1	.....	.....	.04	.....
5 cc. spores heated to 63° C.	71.8	0.2	0.04	.10	0.06
10 cc. spores heated to 63° C.	72.2	.....	.....	.16	.12
20 cc. spores heated to 63° C.	71.9	.1	.02	.20	.16

It will be seen that there was a slight gain in reducing sugars in both 10 and 20 per cent sugar solutions inoculated with spores of *Aspergillus flavus* heated to 63° C. The polarization values are all within experimental error. Upon longer incubation only negligible differences were obtained, which makes it superfluous to record the results. Suffice it to say that one may conclude from the above data that the spores of *Aspergillus flavus* contain very little, if any, invertase.

It is of special interest to consider this problem with regard to the spores *Aspergillus sydowi*, because it has been shown by us [1] that this mold not only occurs with greatest frequency on all types of sugar investigated but furthermore has the greatest deteriorative power of all of the molds isolated. The inoculum used in the present instance contained 600,000 spores per cubic centimeter. The results obtained with it are recorded in Table X.

After three hours' incubation there was a loss of sucrose in both 10 and 20 per cent sugar solutions with 10 and 20 cc. of inoculum. This was further emphasized at the end of two days, when losses of 1.5 and 2 per cent sucrose are to be noted with 20 cc. of inoculum in the 10 and 20 per cent sugar solutions, respectively. The striking fact encountered was that

[1] KOPELOFF, Nicholas, and KOPELOFF, Lillian. OP CIT.

all the cultures inoculated with spores heated to 63° C. were a bluish-gray color, and it appeared that there was considerable matter in suspension. This proved to be a gum, which was precipitated by five volumes of 95 per cent alcohol and came down most abundantly in a strongly alkaline solution. The amount of gum present increased in proportion to an increase in inoculum. It was important to determine whether or not the presence of this gum affected the polarization of the foregoing solutions. Consequently the gum was precipitated from each of two flasks of the 10 and 20 per cent sugar solutions, and the sugar was filtered. The polarization of this filtrate was identical with that of the unfiltered solution, proving that the gum when present in such amount did not influence the polarization value beyond the experimental error of the method employed. The gum was found to exert no influence on Fehling's solution. Further data regarding the properties and nature of this gum are now being obtained and will soon be available for publication.

TABLE X.—*Analyses of 10 and 20 per cent sugar solutions inoculated with spores of Aspergillus sydowi*

10 PER CENT SUGAR SOLUTION

Treatment.	After three hours.			After two days.			Filtrate after alcohol precipitation.		
	Polarization.	Decrease in polarization.	Decrease in sucrose.	Polarization	Decrease in polarization.	Decrease in sucrose.	Polarization.	Decrease in polarization.	Decrease in sucrose.
			Per ct.			*Per ct.*			*Per ct.*
10 cc. sterile water heated to 63° C.	45.8	.....	.....	45.6	.....	.....	44.5	.....	.....
10 cc. sterile water heated to 100° C.	46.0	.....	.....	46.3	.....	.....	44.5	.....	.....
10 cc spores heated to 100° C.	45.5	0.3	0.07	45.6	.....	.....	44.5	.....	.....
5 cc. spores heated to 63° C.	46.1	.....	.....	44.5	1.1	0.27	42.8	1.7	0.43
10 cc. spores heated to 63° C.	45.5	.3	.07	41.6	4.0	1.00	40.3	4.2	1.04
20 cc. spores heated to 63° C.	44.2	1.6	.40	39.7	5.9	1.47	37.4	7.1	1.77

20 PER CENT SUGAR SOLUTION

10 cc. sterile water heated to 63° C.	72.2	.....	.....	72.8	.....	.....	70.5	.....	.....
10 cc. sterile water heated to 100° C.	72.2	.....	.....	72.6	0.2	0.05	70.5	.....	.....
10 cc. spores heated to 100° C.	72.2	.....	.....	72.5	.3	.07	70.5	.....	.....
5 cc. spores heated to 63° C.	72.9	.....	.....	70.9	1.9	.47	68.3	2.2	0.55
10 cc. spores heated to 63° C.	72.1	0.1	0.02	69.0	3.8	.94	67.6	2.9	.72
20 cc. spores heated to 63° C.	70.4	1.8	.44	64.0	8.2	2.05	62.1	8.4	2.10

It was considered advisable to precipitate the gum, filter, and again polarize the solutions after two days' incubation. These values are shown in the last columns of Table X.

The same general relationships as noted in the solutions containing gum were again established. The fact that some evaporation occurred and that these polarizations were made with a 100-mm. tube accounts for the slightly higher values obtained.

After 2½ days' incubation the solutions were again polarized and reducing sugars determined, with the results recorded in Table XI.

TABLE XI.—*Analyses of 10 and 20 per cent sugar solutions inoculated with spores of Aspergillus sydowi*

10 PER CENT SUGAR SOLUTION

Treatment.	After 2½ days' incubation.				
	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 cc. sterile water heated to 63° C.	46. 1	.....	.....	0. 12	.....
10 cc. sterile water heated to 100° C.	46. 2	.....	.....	. 12	.....
10 cc. spores heated to 100° C.	45. 8	0. 3	0. 07	. 12	.....
5 cc. spores heated to 63° C.	44. 4	1. 7	. 42	. 57	0. 45
10 cc. spores heated to 63° C.	40. 9	5. 2	1. 30	. 90	. 78
20 cc. spores heated to 63° C.	38. 6	7. 5	1. 87	1. 24	1. 12

20 PER CENT SUGAR SOLUTION

10 cc. sterile water heated to 63° C.	73. 0	.....	.....	0. 44	.....
10 cc. sterile water heated to 100° C.	73. 0	.....	.....	0. 44	.....
10 cc. spores heated to 100° C.	72. 9	0. 1	0. 02	. 52	0. 08
5 cc. spores heated to 63° C.	71. 0	2. 0	. 50	. 81	. 37
10 cc. spores heatedt o 63° C.	68. 2	4. 8	1. 19	1. 44	1. 00
20 cc. spores heated to 63° C.	63. 5	9. 5	2. 37	1. 80	1. 45

It is at once apparent that there is a striking decrease in sucrose accompanied by an increase in reducing sugars with an increase in the number of spores used for inoculation, and that as with the spores of *Aspergillus niger* the invertase activity is manifest to a greater degree in the 20 per cent than in the 10 per cent sugar solutions.

In order to identify the invertase and gum-forming enzym with the spores of *Aspergillus sydowi* alone, each solution was examined microscopically and plated out in the usual manner on Kopeloff's agar.[1] No contaminating bacteria or other microorganisms were to be found.

Having established that the spores of some molds contain invertase and a gum-forming enzym, it is essential to define the limits of concentration under which these enzyms may operate. Consequently, a 70

[1] KOPELOFF, Nicholas, and KOPELOFF, Lillian. OP. CIT.

per cent (by weight) sugar solution was prepared and diluted to concentrations of 10, 20, 30, 40, 50, and 60 per cent and sterilized in the usual manner. All flasks were inoculated with 10 cc. of spores of *Aspergillus sydowi* containing 120,000 spores per cubic centimeter. The uninoculated flasks received 10 cc. of sterile water heated to 63° C. After 2½ days' incubation the solutions were analyzed as shown in Table XII.

TABLE XII.—*Analyses of sugar solutions of increasing concentration inoculated with spores of Aspergillus sydowi*

Treatment.	After 2½ days' incubation.				
	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 per cent solution, uninoculated	24.0	.....	.....	0.15	.....
10 per cent solution, inoculated with spores at 100° C.	24.0	.....	.....	.15	.....
10 per cent solution, inoculated with spores at 63° C.	22.8	1.2	0.30	.42	0.27
20 per cent solution, uninoculated	48.0	.....	.....	.30	.....
20 per cent solution, inoculated with spores at 63° C.	46.4	1.6	.40	.73	.43
30 per cent solution, uninoculated	72.0	.....	.....	.40	.....
30 per cent solution, inoculated with with spores at 63° C.	71.7	.3	.07	.43	.03
40 per cent solution, uninoculated	96.0	.....	.....	.51	.....
40 per cent solution, inoculated with spores at 63° C.	96.0	.....	.....	.50	.....
50 per cent solution, uninoculated	120.0	.....	.....	.60	.....
50 per cent solution, inoculated with spores at 63° C.	120.0	.....	.....	.60	.....
60 per cent solution, uninoculated	144.0	.....	.....	.80	.....
60 per cent solution, inoculated with spores at 63° C.	143.9	.....	.....	.80	.....
70 per cent solution, uninoculated	168.0	.....	.....	.80	.....
70 per cent solution, inoculated with spores at 63° C.	168.3	.....	.....	.80	.....

It will be seen that inversion occurred in the solutions of 10, 20, and 30 per cent concentration but did not take place in higher concentrations. The formation of gum already described was also found at the former concentrations. Again it will be noted that there was greater inversion with a 20 per cent than with a 10 per cent solution. Unfortunately the inoculation was too meager to produce as large a change as might be desired, and it is altogether likely that an increased inoculation would be more active. Thus, for the inoculum employed, the limit of concentration appears to be between 30 and 40 per cent by weight, which, recalculated to actual percentage of sucrose in the solution, is between 18 and 24 per cent.

The same experiment was repeated, using the spores of *Aspergillus niger* to the extent of 400,000 per cubic centimeter. The result is recorded in Table XIII.

TABLE XIII.—*Analyses of sugar solutions of increasing concentration inoculated with spores of Aspergillus niger*

Treatment.	After 2½ days' incubation.				
	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 per cent solution, uninoculated	24.0	.....	.....	0.14	.....
10 per cent solution, inoculated with spores at 100° C.	24.0	.....	.....	.14	.....
10 per cent solution, inoculated with spores at 63° C.	22.9	1.1	0.27	.32	0.18
20 per cent solution, uninoculated	48.0	.....	.....	.20	.....
20 per cent solution, inoculated with spores at 63° C.	47.4	.6	.15	.29	.09
30 per cent solution, uninoculated	72.0	.....	.....	.33	.....
30 per cent solution, inoculated with spores at 63° C.	71.6	.4	.10	.35	.02
40 per cent solution, uninoculated	96.0	.....	.....	.45	.....
40 per cent solution, inoculated with spores at 63° C.	96.0	.....	.....	.45	.....
50 per cent solution, uninoculated	120.0	.....	.....	.56	.....
50 per cent solution, inoculated with spores at 63° C.	120.0	.....	.....	.57	.....
60 per cent solution, uninoculated	144.0	.....	.....	.70	.....
60 per cent solution, inoculated with spores at 63° C.	144.0	.....	.....	.73	.....
70 per cent solution, uninoculated	168.0	.....	.....	.80	.....
70 per cent solution, inoculated with spores at 63° C.	168.0	.....	.....	.81	.....

It will be observed that inversion occurred to a slight extent in 10, 20, and 30 per cent concentrations but not beyond this point. This corroborates the results previously obtained with the spores of *Aspergillus sydowi* and establishes the upper limit of concentration for the invertase activity of these spores. When recalculated it is found to be between 18 and 24 per cent actual sucrose in the solution.

The query, therefore, which was considered as a basis for this investigation, Do mold spores contain enzyms? has been answered in the affirmative by virtue of the evidence advanced.

It is irrelevant at the present time to do more than indicate the significance of the industrial applications of this biological principle. We have already mentioned that it plays a considerable rôle in the deterioration of sugar by materially affecting the factor of safety rule. It may likewise explain certain transformations in the soil which occur as a result of mold activity where mycelia are not found in great abundance. Thus the universal distribution of molds and their activities will undoubtedly suggest many further developments of this phenomenon, some of which are at present under investigation in this laboratory.

SUMMARY

(1) The spores of *Aspergillus niger*, *Aspergillus sydowi*, and to a lesser extent *Penicillium expansum* and *Aspergillus flavus*, heated to 63° C. for 30 minutes and shaken with sterile sand, caused a decrease in polarization and an increase in reducing sugars in a 10 per cent sterile sugar solution in 3 hours and continued to cause the same changes throughout the 4-day incubation at 45°. Increased activity of a corroborative nature was obtained with 20 per cent sugar solutions. An increase in the number of spores caused an increase in enzymic activity.

(2) The fact that neither spores heated to 100° C. nor an inoculation with sterile distilled water caused any change, indicated the activity mentioned above to be enzymic in nature.

(3) The enzym present exhibited activities indentical with ivertase, consequently the spores of *Aspergillus niger*, *Aspergillus sydowi*, *Penicillium expansum*, and *Aspergillus flavus* contain invertase.

(4) The spores of blue aspergillus contained a gum-forming enzym which paralleled invertase activity.

(5) The limit of concentration of 100,000 to 400,000 mold spores per cubic centimeter for both the invertase activity and the formation of gum was found to be between 18 and 24 per cent actual sucrose.

(6) Among the practical applications of this phenomenon the deterioration of manufactured cane sugar and certain transformations in the soil are especially significant.

NATURE AND CONTROL OF APPLE-SCALD

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NATURE OF APPLE-SCALD

EFFECT OF REMOVAL FROM STORAGE

It is a quite generally accepted idea that apple-scald is due to the warming up of the fruit after it has been removed from cold storage. This idea comes from an erroneous interpretation of very familiar facts (*2*).[1] It is true that apples do not show scald while held continuously in storage at 0°C. (32° F.) and that they seldom show it under commercial cold-storage conditions where the air must from necessity sometimes vary slightly from any desired temperature. It is also true that apples that have been stored for several months in tight packages or closed rooms may show very bad scald after a few days' exposure to warm air. Under such circumstances, it is natural to conclude that the warming up of the fruit is the cause of the scald; but the facts of the case are that the apples are already potentially scalded, and the higher temperatures merely allow the death processes of the apple tissue to be carried out. The real cause of the disease is to be found in the conditions of transportation and storage to which the fruit is subjected during the first few weeks after it is removed from the tree. Healthy apples do not develop scald upon removal from cold storage, even when transferred at once to living-room temperatures.

RELATION OF THE COMPOSITION OF THE STORAGE AIR TO APPLE-SCALD

The fact that outside air produces such serious results on potentially scalded apples has led to a belief that apples should be kept away from fresh air and air currents as much as possible at all times; but carefully controlled storage experiments have shown conclusively that when the fruit is stored in open packages, scald can be entirely prevented by thorough stirring of the storage air. The question naturally arises as to the nature of the harmful substances that are carried away from the fruit by this air movement.

HUMIDITY.—Many storage men hold the opinion that excessive moisture may bring about apple-scald. The writers have made a number of carefully controlled experiments with apples held under different humidities and, as reported later, have also followed up the moisture conditions

[1] Reference is made by number (italic) to "Literature cited," p. 240.

in commercial cold storage. The results indicate that when moisture remains condensed in drops on the fruit there may be a slight increase in the development of scald, but apparently because of the more restricted aeration of the apple tissue rather than from any harmful effect of the water itself. Apples stored in air that was saturated with moisture but constantly stirred have not developed scald, while similar apples in dry, stagnant air have become badly scalded. Apples have been exposed to outside air by throwing a cold-storage room open freely when the temperature would allow, and by rolling the barrels out of the storage room for one or two days each week; but scald has been reduced rather than increased by the treatment. (See p. 223.) Apples that have been picked wet or have had water poured over them after they were in the barrel have developed no more scald than others picked and packed when they were dry. The writers have found no evidence that excessive humidity plays any important part in the development of scald either under experimental or commercial storage conditions.

OXYGEN.—The apple is a breathing organism, and under conditions of restricted aeration the percentage of oxygen in the air surrounding the fruit is reduced below normal. The question naturally arises as to whether this change in air composition has any influence upon the development of scald. To test this point, apples were stored in air which had the percentage of oxygen reduced from 21 to 6.9, and others in air which had the percentage of oxygen increased to 31.5. The amount of scald developed in each lot was compared with the amount on apples held in air having the normal percentage of oxygen. The reduced oxygen supply resulted in no increase in the amount of scald, and the increased oxygen supply gave no significant decrease in scald. In other experiments, Grimes apples were held in 100 per cent oxygen at 20° C. for four days and then removed from the oxygen and placed in moist chambers in normal air, part of the apples being then stored at 15° and part at 2.5°. Other apples were exposed to the same temperatures but were not given the preliminary oxygen treatment. Notes were taken at various times on the amount of scald, but no difference of any kind developed between the apples that had been first stored in oxygen and those that had not. The results are strikingly different from those reported later from similar experiments with carbon dioxid. (See p. 213.) The results of the various experiments seemed to prove conclusively that the small variations in the oxygen content that ordinarily occur in the storage air are not matters of importance in determining the development of scald.

OZONE.—Although an increased oxygen supply resulted in little or no decrease in the amount of scald, it seemed possible that a more powerful oxidizing agent might give different results. So in the fall of 1918 both laboratory and commercial cold-storage experiments were made with ozone.

In the laboratory tests a small but powerful ozone machine was used. In the preliminary experiments the ozonated air was used in its full strength as it came from the machine; but it injured the apples, soon producing brown dead spots at the lenticels. In the final experiments the air from the ozone machine was reduced to half strength by mixing with an equal volume of normal air. The apples used in the tests were Grimes from Vienna, Va. They were picked August 21 and the experiments started the following day. The fruit was stored in 8-liter jars that were fitted with 2-hole stoppers. Once every week ozonated air was drawn through these jars, the incoming current being freed at the bottom of the jars and the outgoing current taken from the top. The process was continued for 5 minutes and the jar then tightly corked and allowed to stand closed for the next 24 hours, after which the 5/8-inch stopper was removed and the jar left as a moist chamber for the remainder of the week. At the end of the ozone treatment the air from the exit tubes had a strong smell of ozone, but after 24 hours no ozone odor could be detected in the air of the jars. Part of the apples were stored at 15° and part at 0° C. In each experiment, control jars of apples were maintained that were identical with the others in every respect, except that in the weekly treatment normal air was drawn through instead of the ozonated air. Notes were taken at various times on the amount of scald and on the quality of the fruit, but no contrast of any kind was found between the apples that were treated with ozone and those that were not.

In the commercial cold-storage experiments, the ozone was obtained from a large 12-cylinder machine of the type most commonly used in egg storage rooms. The machine was operated from six to eight hours a day and from five to seven days a week. Six barrels of Grimes apples and six barrels of York Imperial were stored within a few feet of the machine, while similar lots were held as controls in another storage room. In each test, half of the apples were in ventilated barrels and half in barrels of the usual commercial type. The final notes on the Grimes apples were taken December 20 and the final notes on the York Imperial on January 18. Both varieties had scalded badly; but there was no contrast, in either the ventilated or unventilated barrels, between the fruit that had been exposed to ozonated air and that which had not. The results give little promise of scald prevention by increased oxidation.

Carbon dioxid.—Carbon dioxid is the gas produced in greatest quantity by storage fruit and is therefore the one that might most naturally be expected to produce harmful results. Experiments reported in an earlier paper (*2*), however, have shown that apples stored continuously in atmospheres having percentages of carbon dioxid similar to those in commercial storage, or even considerably exceeding them,

have shown no sign of injury and have developed less scald than similar apples held in air that was free from carbon dioxid. It was also found that apples could be made less susceptible to scald by storing them for a few days in an atmosphere composed entirely of carbon dioxid but that this treatment sometimes gave the apples a disagreeable alcoholic taste. In order to get further data as to the carbon-dioxid endurance of the apple, these latter experiments were repeated in the fall of 1918 with the period of storage in carbon dioxid shortened. The results are given in Table I. Lots A, B, and C were Grimes from Vienna, Va. They were picked August 20 and the experiment started the following day. The apples in lot D were Grimes from Wenatchee, Wash. They were shipped to Washington, D. C., in a pony refrigerator and the experiment started September 20. Lot E was Yellow Newtown apples from Winchester, Va. They were in cold storage until December 19, the date of starting the experiment. In all the tests the apples receiving prestorage treatment with carbon dioxid were removed from this gas at the end of the given number of days and stored in moist chambers in normal air. Thereafter they had the same air and moisture conditions as the controls had from the beginning of the experiment.

TABLE I.—*Effect of prestorage treatment with carbon dioxid upon the development of apple-scald*

Lot.	Prestorage conditions.		Storage temperature.	Number of weeks in storage.	Percentage of scald.	
	Temperature.	Number of days held.			Apples held in 100 per cent carbon dioxid during prestorage period.	Apples held in normal air during prestorage period.
	°C.		°C.			
A	30	2	10	10	10	52
B	20	4	15	10	35	60
C	20	4	2½	16	4	38
D	15	2	5	13	10	50
D_1	15	6	5	13	6	.....
D_2	30	2	5	13	0	.....
E	15	6	0	15	0	15

At the end of the various experiments the apples that had been treated with carbon dioxid were slightly greener than the untreated fruit but were apparently normal in taste and general appearance. A study of the last two columns of the table will show that the apples exposed to carbon dioxid developed much less scald than those that were not, giving further evidence that carbon dioxid has no tendency to produce the disease. The writers are of the opinion that the apparently beneficial effects of the carbon dioxid treatment are due to a general checking of the skin activities of the apple rather than to any specific

favorable effect upon apple scald. The results of these and earlier experiments show, however, that it is possible to use carbon dioxid as an agency for reducing apple scald and that this can be accomplished without evident injury to the apple.

While it seems to have been conclusively proved that carbon dioxid is not responsible for the occurrence of apple scald, it does not follow that high percentages of the gas in storage air are to be looked upon with favor, for such a condition would indicate a lack of air movement and an accumulation of other gaseous products of the apple as well as of carbon dioxid.

ARTIFICIAL SCALD.—Various attempts have been made to produce apple-scald artificially or to shorten its period of development by changing the composition of the air; but, as mentioned in the discussions of humidity and carbon dioxid, these have usually met with failure. Other experiments of this sort were made with alcohols, acids, and esters. In the preliminary tests the various substances were used in full strength and were placed in close proximity to the fruit, but this led to such serious and rapid injury from the more active agents that nothing resembling scald was produced. In the later experiments dilutions were made as indicated below, and the liquids were placed in the bottom of 8-liter jars with the apples supported in the top at a distance of approximately 12 inches from the chemicals. Twenty-five cc. of the material were used in each jar. All the jars were loosely stoppered. The experiments were made at 10° C. The results reported below are based on the appearance of the apples after they had been removed from the storage condition and had stood in a warm laboratory for 24 hours.

TABLE II.—*Effects of various volatile substances on Yellow Newtown and Rome Beauty apples*

Substances used.	Effects.
Water, control	Apples stored over water developed no scald or other injury.
Ethyl alcohol	At the end of 3 weeks no scald had developed, and the apples were apparently still normal.
Acetic acid	Rome Beauty apples showed injury at the end of 24 hours, but the effects did not resemble scald.
Alcohol 60 per cent, acetic acid 40 per cent.	Rome Beauty apples had their flesh killed and browned to a depth of ⅛ inch at the end of 48 hours, but the effects did not resemble scald.
Alcohol, 90 per cent, acetic acid, 10 per cent.	At the end of 7 days dead brown areas of various patterns were scattered over the skin of the apple. Many of the smaller spots were located at the lenticels. There was a clear-cut margin between the diseased and the healthy areas, and the flesh was affected to a much greater depth than it would be by scald. The brown spots were as common on the highly colored portions of the skin as on the poorly colored ones. There was but slight resemblance to typical scald.
Formic acid 100 per cent	No scald or other injury after 3 weeks.

TABLE II.—*Effect of various volatile substances on Yellow Newtown and Rome Beauty apples*—Continued

Substances used.	Effects.
Alcohol 80 per cent, ethyl acetate 20 per cent.	After 3 days' exposure to the vapors, Rome Beauty apples had a brown, cooked appearance, the red portions of the apples, however, being much less affected than the green portions.
Alcohol 90 per cent, ethyl acetate 10 per cent.	After 7 days, Rome Beauty and Yellow Newtown apples had the appearance of being typically scalded. The scalded areas occurred only on the green side of the fruit and shaded off in severity as the blush areas were approached. After standing in a warm room for 4 days the browning had spread into the flesh of the apple rather more rapidly than is usual with scald, but aside from this the diseased condition was typical of apple-scald.
Alcohol 80 per cent, amyl acetate 20 per cent.	Only Rome Beauty apples were tested. The results were practically the same as with 10 per cent ethyl acetate, a quite typical scald being produced.
Alcohol 90 per cent, amyl acetate 10 per cent.	Experiments were made with both Rome Beauty and Yellow Newtown apples. The former were removed at the end of 3 days and the latter at the end of 7 days. The results were similar to those reported for 10 per cent ethyl acetate, but the apples were even more typically scalded. The browned areas coincided in the most exact manner with the skin areas naturally susceptible to scald.
Ethyl malate 10 per cent.....	Only about 10 cc. of the liquid were used, and the apples were placed in a smaller jar than those mentioned above. No scald or other injury had been produced at the end of 3 weeks.
Ethyl butyrate.....	Experiments somewhat similar to those described above gave results very much like those obtained with ethyl acetate and amyl acetate.

The results reported in Table II show that it is possible to produce an apparently typical apple-scald within a few days by exposing the fruit to the vapors of certain dilute esters. The fact that the disease can be thus produced artificially when connected with the additional fact that the apples themselves are known to give off esters or related gases makes it seem probable that these substances play an important part in the development of scald as it occurs in commercial storage.

GAS ABSORBENTS AS SCALD PREVENTIVES

In an earlier paper (5) experiments were reported showing that scald can be practically prevented by wrapping the apples in paper that has been infiltrated with fat or oil. Other experiments are reported later in the present paper (see p. 233) that give full confirmation of these results and also make it clear that the beneficial effects of the wrappers are not due to modifications in the moisture or the carbon-dioxid content of the air surrounding the apple. It is well known that fats and oils have a great absorbing power for esters and other odorous gases, and because of this property they are used commercially in the extraction of perfumery. Cow butter takes up odors so readily that it is usually rendered unpal-

atable if held in storage with other food products. In view of these facts there seems to be little doubt that the beneficial effects of the fats and oils in the wrappers are due to the absorption of esters or similar products thrown off by the apple. The hypothesis is given further support by the fact that the fats and oils which are known to have a great absorbing power for gases give more complete control of scald than paraffin and similar waxes that are generally recognized as being more inactive toward these gases, and also by the results in the experiments reported above in which scald was produced artificially by exposing apples to various esters.

It is generally recognized in the apple trade that greasy, waxy apples do not scald as soon or as badly as the others. The above hypothesis offers a possible explanation for this fact, the wax of the apple, like that in the wrapper, apparently serving as an absorbent for the harmful gases.

TISSUES AFFECTED BY APPLE-SCALD

Scald is typically a skin disease of the apple. In the early and more typical stages of the trouble, only the five or six surface layers of cells that form the color-bearing tissue of the apple are affected. With long continued unfavorable conditions the apple tissue may become dead, brown, and rotlike to a depth of ⅛ to ¼ inch, and occasionally the disease spreads practically to the core. Reference is made here to the scald itself; but with the death of the protective skin layer, various rot organisms have free access to the softer tissues beneath and usually play an important part in hastening the destruction of the apple. Not all portions of the skin are equally susceptible to scald. The highly colored areas of red apples are affected only in the most extreme cases of scald. Often when the poorly colored areas are badly and deeply scalded, the diseased condition will shade off into a mere brown tint of the skin as the margin of the blush area is approached. The chemical changes that occur in the reddening of the fruit apparently produce a skin condition that is highly resistant to scald.

The statement is quite generally current that apples that still show the leaf green are very much more susceptible to scald than those which have become slightly yellowed. As a rough statement of the facts, this may be approximately true. The observations of the writers, however, indicate that while green apples, in general, are more susceptible to scald than ripe ones, those still having the leaf green are very much less susceptible than those that have just begun to turn yellow, and often less susceptible than those in which the ground color has become a deep yellow. They have also observed that while green apples may finally become more severely scalded than riper ones, the latter usually scald first if they develop the disease at all. In spite of these qualifications, it is still true that scald can be greatly reduced and delayed by leaving the apples on the tree till well matured.

INFLUENCE OF ORCHARD CONDITIONS

It is generally admitted that the susceptibility of apples to scald probably varies with orchard conditions, but little experimental data has ever been published on the subject. In the fall of 1918 apples were secured from various soil and orchard conditions at Wenatchee, Wash., for comparative storage tests on this point. In one test, Grimes apples from lightly irrigated trees growing in heavy clay soil were compared with similar apples from lightly irrigated trees on alluvial sand receiving a spring application of 10 pounds of sodium nitrate per tree. The apples were stored in the usual box packages in commercial cold storage. They were removed to a temperature of 15° C. (59° F.) on February 6 and notes taken February 15. The apples from the heavy clay soil had 35 per cent of scald and those from the heavily fertilized sandy soil 60 per cent of scald.

Experiments were conducted also with apples from a Grimes orchard in which irrigation experiments were being made. The orchard was in alfalfa and the soil of the various plots was quite uniform. The contrasts in irrigation were started the first of July and maintained for the rest of the season. With the heavily irrigated plot the soil moisture was kept at approximately 50 per cent of saturation, and with the lightly irrigated one at approximately 20 per cent of saturation, while with the third plot the soil moisture was kept at approximately 20 per cent from July 1 to August 15, and then at approximately 50 per cent the remainder of the season. The methods of irrigation, soil sampling, etc., were the same as those reported in an earlier paper (*4*) and will not be repeated here. The apples were picked on September 18, when mature but not overripe, and placed in storage the following day. They were removed from storage February 5 and were held at a temperature of 15° C. (59° F.) for one week before notes were taken. In experiment A one box of apples from each plot was used under each storage condition. In experiment B separate records were made for the apples of different sizes. There were from 1 to 6 pecks of each size under each storage condition. The results are given in Table III.

The apples from the plot receiving light irrigation early and heavy irrigation late developed about twice as much scald as those from the plot receiving heavy irrigation continuously and three to four times as much as those from the lightly irrigated plot.

It is particularly interesting to note that the increased amount of scald on the heavily irrigated apples was not due to their larger size, since the increase was as great on the small apples as on the large ones. It has been observed in an earlier publication (*5*) that large apples often scald worse than small ones. The foregoing results indicate that in the present case size is a secondary factor, the real cause of the increased scald being

some forcing agency, such as heavy irrigation, that has apparently rendered both large and small apples more susceptible to the disease.

TABLE III.—*Effect of orchard irrigation upon the development of scald in storage: Experiments with Grimes apples at Wenatchee, Wash., 1918*

Kind of storage.	Irrigation.	Percentage of scald.				
			Experiment B.			
		Experiment A.	Apples 2¾ inches and smaller.	Apples 2¾ to 3 inches.	Apples 3 to 3¼ inches.	Apples 3¼ to 3½ inches.
Cellar	Heavy	83	92	88	90	73
	Light	22	23	21	15	No apples.
	Light followed by heavy	50	.....	.....	.....	.....
Air-cooled	Heavy	59	42	56	64	75
	Light	17	14	19	5	No apples.
	Light followed by heavy	40	.....	.....	.....	.....
Cold	Heavy	69	53	79	75	61
	Light	23	6	27	28	No apples.
	Light followed by heavy	57	.....	.....	.....	.....

RELATION OF TEMPERATURE TO THE OCCURRENCE OF APPLE-SCALD

The temperature relations of apple scald have been rather fully discussed in earlier reports (*5*). The rate of scald development increases with a rise in temperature; between 0° and 20° C. each rise of 5° hastens the time of scald appearance by two to six weeks, the greatest contrast occurring between 5° and 10° and the least between 15° and 20°. At 0° scald does not become evident. The apples become latently or potentially scalded but give little evidence of it until removed to a warmer temperature. At temperatures of 25° and above it has not been found possible to produce apple-scald, although other physiological troubles, such as internal breakdown, have developed all the more rapidly at these temperatures. Scald has been greatly delayed and in some cases apparently entirely prevented by bringing apples out after four or five weeks in commercial cold storage and giving them a thorough airing for 24 hours at a temperature of 22°. The hypothesis that scald is due to the accumulation of apple esters may furnish at least a partial explanation for the peculiar effects of the higher temperatures. The fruit esters are in general quite volatile, and their rate of vaporization is greatly increased by a rise in temperature. It seems possible that the slight increase in the rate of scald development in passing from 15° to 20° and the absence of the disease at 25° and 30° may be partly if not entirely due to the greater vaporization of the harmful products at these higher temperatures. It should not be overlooked,

however, that there is a marked change in the general ripening processes of the apple at the higher temperatures and that there may be much more fundamental reasons than the one suggested above for the absence of scald at these temperatures.

EXPERIMENTS IN THE CONTROL OF APPLE-SCALD

In the fall of 1918 apple storage experiments were started in Wenatchee, Wash., Winchester, Va., and Washington, D. C. In the smaller lots the apples were carefully selected from the tree, and in the larger lots they were taken as they came from the packing table. In obtaining records on the degree of scald the maximum scald that had been observed on the variety was taken as 100, and the amount of scald in a particular case was determined by its relation to this standard. Consideration was given to the area and depth of the scald as well as to the number of apples affected.

As previously mentioned (p. 211) apples may be potentially or latently scalded and yet not show it while held continuously at 0° C. (32° F.). In order to get the actual condition of the fruit as it came from storage, it was therefore held at a temperature of 20° C. (68° F.) for three days before the final notes were taken.

RELATION OF MATURITY OF FRUIT TO APPLE-SCALD

Powell and Fulton (*8*) were apparently the first to call attention to the importance of the maturity of the fruit in the control of apple-scald. Beach (*1*), Greene (*6*), Markell (7), Ramsey (*9*), and others have published confirmatory data. As pointed out earlier in this article (p. 217), the writers have found some definite exceptions to the rule that green fruit scalds worse than ripe, but in general their experimental data support the work of earlier investigators.

Table IV gives the results obtained with early and late pickings of Grimes, Rome Beauty, and York Imperial apples. The fruit was stored promptly in all tests. The Grimes and Rome Beauty apples were held in air-cooled cellar storage at Wenatchee, Wash. During September the average temperature of the cellar was 15° C. (59° F.), and the average humidity 75 per cent; during October the average temperature was 11° C. (51.8° F.), and the average humidity 72 per cent; and for the remainder of the storage period the average temperature was 2.5° C. (36.5° F.), and the average humidity 82 per cent. There was a daily fluctuation of from 2° to 4° C. The data given in the table were obtained after the apples had been held at 20° C. (68° F.) for nine days. The percentages in each case are practically double those recorded at the time of removal from cellar storage. One box of apples was used from each picking. The York Imperial apples were held at 0° C. (32° F.) in direct expansion commercial cold storage at Winchester, Va. Three barrels of apples were used under each condition in each test.

All the apples were practically free from scald when removed from storage. The data reported were obtained after the fruit had been held at 20° C. (68° F.) for three days.

TABLE IV.—*Relation of maturity of fruit to apple-scald*

Variety and location.	Package.	Date of picking.	Condition of fruit.	Date of note taking.	Weeks in storage.	Percentage of scald.
Grimes at Wenatchee, Wash.	Box	Sept. 7	Immature	Feb. 15	23	52
		Sept. 17	Mature commercial picking.	...do	22	30
		Oct. 2	Ripe	...do	19	18
Rome Beauty at Wenatchee, Wash.	.....do.....	..do.....	Green	Mar. 15	23	20
		..do.....	Well-colored	...do	23	6
		Oct. 21	Ripe to overripe	...do	21	0
York Imperial at Winchester, Va.	Commercial barrel.	Oct. 1	Rather immature	Jan. 28	17	28
		..do.....	.....do.....	Feb. 25	21	45
		Oct. 29	Mature	...do	17	25
Do	Ventilated barrel.	Oct. 1	Rather immature	Jan. 28	17	11
		..do.....	.....do.....	Feb. 25	21	18
		Oct. 29	Mature	...do	17	0

In all tests the early picked fruit developed more scald than the late picked. A study of the results on York Imperial might indicate that this was largely due to the fact that the early picked apples had been in storage longer. To get a fair test of the relative susceptibility of the two pickings to scald, the February 25 data on the October picking should be compared with the January 28 data on the October 1 picking, thus giving an equal storage period (17 weeks) for each lot. This method of comparison greatly reduces the contrast between the early picked and the late picked fruit, but the latter still maintains a superiority in scald resistance. The great difficulty of scald control by means of maturity lies in the fact that it is often impracticable to leave the fruit on the tree late enough to secure the desired results.

AERATION AS A PREVENTIVE FOR APPLE-SCALD

It has been proved by carefully controlled experiments that apple-scald can be completely prevented by giving the fruit sufficient aeration. This is readily accomplished with small lots of fruit in experimental storage, and the following experiments indicate that the principle can be used to advantage under commercial storage conditions.

AERATION IN DELAYED STORAGE.

There is no period in the storage life of the apple when aeration is so important as in the first week or two after the fruit is removed from the tree, especially in cases where it is impossible to hold it at low temperatures. Table V gives the results of several experiments in delayed storage. Three barrels or three boxes of fruit were used under each condition of each test. They were all held in commercial cold storage

but were removed to a temperature of 20° C. (68° F.) and held for three days before the final notes were taken. The apples used were as follows:

A, Grimes from Franklin, Va.; picked August 30; final notes taken December 20.

B, Rome Beauty from Franklin, Va.; picked September 27; final notes taken January 28.

C, York Imperial from Greenwood, Va.; picked October 10; final notes taken January 31.

D, Grimes from Winchester, Va.; picked September 10; final notes taken January 16.

E, Stayman Winesap from Winchester, Va.; picked September 25; final notes taken January 22.

F, York Imperial from Winchester, Va.; picked October 1; final notes taken February 11.

G, Rome Beauty from Wenatchee, Wash.; picked October 2; final notes taken March 25.

TABLE V.—*Effect of delayed storage and of aeration during delay*

Variety.	Treatment.	Percentage of scald.		
		Commercial barrel.	Ventilated barrel.	Box.
A, Grimes.	Immediate storage	35	30	.....
	Delayed 10 days in open packing shed	12	3	.....
	Delayed 10 days in sun in boxes	12	.....	.....
B, Rome Beauty.	Immediate storage	28	20	.....
	Delayed 10 days in warm laboratory, temperature 21.1° to 23.9° C. (70° to 75° F.).	80	0	.....
C, York Imperial.	In transit, by express, 3 days	20	8	.....
	In transit, by freight, 15 days	60	17	.....
D, Grimes.	Immediate storage	58	12	.....
	Delayed 9 days in closed packing shed, temperature 15.5° to 23.9° C. (60° to 75° F.).	47	12	.....
E, Stayman Winesap.	Immediate storage	50	16	.....
	Delayed 6 days in hall of cold-storage plant, temperature 10° to 12.8° C. (50° to 55° F.).	70	12	.....
	As above but delayed 10 days	70	15	.....
F, York Imperial.	Immediate storage	35	25	.....
	Delayed 8 days in hall of cold-storage plant, temperature 12.8° to 15.5° C. (55° to 60° F.).	50	30	.....
	As above but delayed 15 days	65	35	.....
G, Rome Beauty.	Immediate storage	.....	.....	12
	Delayed 9 days in the open, in the shade, temperature 5.6° to 15° C. (42° to 59° F.).	.....	.....	0
	Delayed 9 days in a closed room, temperature 7.2° to 12.8° C. (45° to 55° F.).	.....	.....	7

If a study is made of the results in the first column, it will be seen that in four out of six tests scald was greatly increased by delayed storage, and in the other two (A and D) it was quite definitely decreased. The tests in which there was a decrease are those in which the apples received the greatest amount of aeration during the delay. With the

ventilated barrels, scald was greatly decreased by delay in two of the tests, decidedly increased in one, and apparently but little affected in the others. With the boxes, scald was slightly decreased by delay in a closed room and entirely prevented by delay in the open. The most striking feature of the table, however, is seen when the scald in the delayed, ventilated barrels is compared with that in the immediate storage, commercial barrels. The good effects of the more open package have far more than offset any bad effects from the delay, and have resulted in reducing the scald to about one-fourth of that on the immediately stored fruit in the unventilated or commercial barrel. Delayed storage may evidently be either favorable or unfavorable to the development of scald, depending upon the conditions under which the fruit is held. If it is possible to give good aeration during the delay, the results may be distinctly beneficial to the fruit, especially if it is rather immature; but as is shown in Table V, delay in closed rooms or in unrefrigerated cars is likely to result in the development of serious scald later in storage.

TEMPERATURE CHANGES AS A MEANS OF AERATION

It is generally believed that changes in the temperature of the fruit or the storage room are likely to produce serious results. The experiments reported in Tables VI, VII, and VIII indicate that so far as apple-scald is concerned, temperature changes may sometimes prove beneficial.

The apples used in Table VI were Grimes from Vienna, Va. They were stored September 3, and notes were taken December 20. Two barrels of apples were used under each condition. The laboratory to which part of the apples were removed stood at a temperature of 20° C. (68° F.), and the apples were held there for 24 hours at a time. The hall into which other apples were rolled had an open window but was protected from outside winds. The temperature was from 2½° to 5° C. (4½° to 9° F.) warmer than that of the storage room. The apples were left in the hall for about 24 hours at a time.

In the experiment reported in Table VII the apples were from Wenatchee, Wash. The Rome Beauty apples were stored October 2 and the Stayman Winesap October 12. The notes on both were taken March 25. The storage room stood at 0° C. (32° F.). The engine room to which part of the apples were moved had a temperature of 14.4° C. (58° F.) during the time of the first airing, and a temperature of 12.8° C. (55° F.) during the second airing. The average temperature of the outside air during the first airing was 7.8° C. (46° F.), and at the time of the second airing 8.8° C. (48° F.). One box of apples was used under each condition.

In the experiment reported in Table VIII the apples were from Winchester, Va. The Arkansas apples were stored October 28, and the notes taken February 3; the Stayman Winesap stored September 25, and the

notes taken January 23; the York Imperial stored October 1, and the notes taken February 11; the Yellow Newtown stored October 25, and the notes taken April 7. Three barrels of each variety were left continuously in storage and three rolled into the hall for a 24-hour period once each week during the first 2½ months of storage. The temperature of the storage room stood at 0° C. (32° F.) or slightly above; during October the hall had a temperature of 7° to 10° C. (44.6° to 50° F.), and in the later months a day temperature of about 5° C. (41° F.) and a night temperature of approximately 0° C. (32° F.) The hall doors were kept open, giving free circulation of outside air. Resistance thermometer bulbs were forced into apples in the center of the barrels and temperature readings taken when the apples were removed to the hall and again when they were returned to storage. The temperature of the fruit was never raised more than 1° C. (1.8° F.) by exposure to the hall temperature for 24 hours. In all the different tests the apples were held at 20° C. (68° F.) for three days before the notes were taken.

TABLE VI.—*Effect of temperature changes upon apple-scald: Experiment at Washington, D. C.*

Lot No.		Percentage of scald.	
		Grimes in commercial barrel.	Grimes in ventilated barrel.
1	In cold storage continuously 16 weeks	35	30
2	As in 1 but at 20° C. (68° F.) 1 day at the end of 6 weeks' storage	31	18
3	As in 1 but at 20° C. (68° F.) 1 day each at the end of 6 and 11 weeks' storage	25	5
4	As in 1 but at 20° C. (68° F.) 1 day at the end of 11 weeks' storage	38	35
5	As in 1 but in cold-storage hall 1 day each at the end of 5, 7, and 11 weeks	.....	10
6	As in 1 but in cold-storage hall 1 day each at the end of 7 and 11 weeks	35	5
7	As in 1 but in cold-storage hall 1 day at the end of 7 weeks	28	12
8	As in 1 but in cold-storage hall 1 day at the end of 11 weeks	35	23

TABLE VII.—*Effect of temperature changes upon apple-scald: Experiment at Wenatchee, Wash.*

Lot No.		Percentage of scald in boxes.	
		Rome Beauty.	Stayman Winesap.
1	In cold storage continuously	11	12
2	In engine room for 6 hours on Nov. 7 and again for 6 hours on Dec. 2	14	8
3	As in 2 but removed to the open air for the same 6-hour periods	9	13

TABLE VIII.—*Effect of temperature changes upon apple-scald: Experiment at Winchester, Va.*

Lot No.	Treatment.	Percentage of scald in commercial barrels.			
		Arkansas.	Stayman Winesap.	York Imperial.	Yellow Newtown.
1	In cold storage continuously	50	67	45	10
2	Moved from cold storage to storage hall 1 day each week during the first 2½ months of storage	30	34	45	12

No harmful effects of any kind were found to result from the exposure of the apples to outside air. A study of the tables shows that scald was either not affected or else was reduced by the treatment, the results apparently depending upon the amount of aeration the apples received while out of storage. In the experiment reported in Table VI, where the commercial barrels were removed to rather poorly ventilated rooms 1 to 3 times, the treatment had practically no effect upon scald, but in the experiment reported in Table VIII, where similar barrels were removed to a well-ventilated hall 8 to 10 times, scald was considerably reduced. While the aeration reported in Table VI was apparently too slight to affect the apples in the commercial barrels, the same treatment resulted in a decided reduction of scald in the ventilated barrels, the difference apparently being due to the better aeration secured by the more open package. It is interesting to note in Table VI that while the aerations given at the end of the seventh week of storage decidedly decrease scald, those at the end of the eleventh week had but little effect upon the disease. This is in agreement with data reported in an earlier publication (5), indicating that with Grimes apples aerations must be made during the first 8 or 9 weeks of storage in order to have any beneficial effect upon scald.

The barrels removed from the storage rooms were exposed to more breezes than those that remained, but the aeration received by the apples which were moved was doubtless greatly increased by the air currents set up as a result of the difference between the temperature of the fruit and that of the outside air.

AIR-COOLED STORAGE

Experiments were made to determine the comparative development of apple-scald in air-cooled and cold-storage plants. The results are given in Tables IX and X. The apples used in the experiment recorded in Table IX were from Winchester, Va. Three barrels of each variety were used under each condition. The Arkansas apples were stored October 18, and the final notes taken February 3; the Yellow Newtown stored October 25,

and the final notes taken April 7; and the York Imperial stored October 29, and the final notes taken February 20. All the apples were in direct expansion cold storage from the time of storing till October 31 and were therefore well cooled before being placed in the air-cooled storage. The direct expansion storage house was located at Winchester, Va., and the air-cooled plant at Gerrardstown, W. Va. Hygrothermograph records were kept for both plants throughout the storage season. In the direct expansion rooms, the temperature was held at 0° C. (32° F.) or slightly above; and the relative humidity ranged from 65 to 90 per cent, standing between 75 and 80 per cent during most of the storage season. In the air-cooled plant, the temperature ranged from 5° to 15° C. (41° to 59° F.) during the period from October 31 to November 20 and throughout the remainder of the storage period was fairly constant at 5° C. (41° F.), seldom varying from this temperature more than 1° in either direction. The relative humidity in the air-cooled plant ranged from 40 to 90 per cent, the daily variations often covering a large part of this range. The average relative humidity was approximately 65 per cent.

TABLE IX.—*Apple-scald in air-cooled storage: Experiment at Winchester, Va.*

Lot No.	Treatment.	Percentage of scald.			
		Ventilated barrels.	Commercial barrels.		
		York Imperial.	York Imperial.	Arkansas.	Yellow Newtown.
1	Cold storage, in aisle	1	25	50	10
2	Air-cooled storage Oct. 31 to Dec. 17, and cold storage the rest of the storage period	.....	45	60	.....
3	Air-cooled storage Oct. 31 to Nov. 26, and cold storage the rest of the storage period	3	25	55	35
4	Air-cooled storage Nov. 26 to Dec. 17, and cold storage the rest of the storage period	1	40	30	20

The experiments recorded in Table X were made in Wenatchee, Wash. The Grimes apples were picked September 18, and the Yellow Bellflower September 21. The notes on both were taken February 15. The Rome Beauty apples were picked October 2, and the Stayman Winesap October 4. Both were removed from storage March 17 and notes taken March 25. One box of each variety was used under each storage condition. The average temperature of the cold-storage plant was 5° C. (41° F.) during September and October, 1.8° C. (35.2° F.) during November, and 0° C. (32° F.) during the remainder of the storage period. The relative humidity ranged between 80 and 90 per cent, averaging approximately 85 per cent for the entire storage period. In the air-cooled plant the average temperature during September and October was 12.2° C. (54° F.),

during November 3.3° C. (38° F.), during December 2.2° C. (36° F.), and for the remainder of the storage period 0.8° C. (33.4° F.). In the air-cooled cellar the temperatures were those given in paragraph 4, page 220. All the apples were moved to a temperature of 20° C. (68° F.) one week before the final notes were taken.

TABLE X.—*Apple-scald in air-cooled storage: Experiments at Wenatchee, Wash.*

Lot No.	Treatment.	Percentage of scald in boxes.					
		Grimes.	Rome Beauty.	Stayman Winesap.	Yellow Bellflower.	Grimes.	
						Heavily irrigated.	Lightly irrigated.
1	Cold storage	35	11	12	15	69	23
2	Air-cooled storage	30	6	.....	0	59	17
3	Cold storage 1 month, then air-cooled storage	42	.....	4	.....	.....	.....
4	Air-cooled storage 1 month, then cold storage	7	.....	0	.....	.....	.....
5	Cold storage 2 months, then air-cooled storage	40	10	[a]14	...	.....	.....
6	Air-cooled storage 2 months, then cold storage	60	7	*b*0	.....	.....	
7	Air-cooled cellar storage	.....	.....	.....	.....	83	22

[a] Cold storage 4 months. [b] Air-cooled storage 4 months.

In the West Virginia experiment, the apples in air-cooled storage scalded worse than those in cold storage, while in the experiment at Wenatchee, Wash., the apples in cellar storage were scalded most, the ones in cold storage next, and those in air-cooled storage least. The results appear to be contradictory, but are really in harmony with the fundamental facts. It was pointed out earlier in the paper that scald development is decreased by low temperatures and also by aeration. The temperatures in the cellar storage at Wenatchee and in the air-cooled plant at Gerrardstown, W. Va., were higher than those in the air-cooled plant at Wenatchee; and the air circulation in the first two places was also poorer than that in the last. So while the results reported in the table appear contradictory so far as air-cooled storage is concerned, they are in harmony with the laws of scald occurrence. Air-cooled storage conditions vary greatly with the weather and with the construction and management of the storage house, and the results on scald will necessarily vary accordingly. The infrequency of cool nights in the fall of 1918 made the management of air-cooled houses unusually difficult.

COLD-STORAGE SYSTEMS AND METHODS

Experiments were made at Wenatchee, Wash., and Winchester, Va., to determine the effect of ventilation and aeration in commercial cold-storage plants. The apples were held at 0° C. (32° F.) or slightly above

in all the different tests. In the Wenatchee experiment part of the apples were stored in a room cooled by direct expansion and the others in a room cooled by the bunker system. In the former experiment there was practically no air movement, while in the latter the apples were stored at a distance of about 3 feet from an opening in the outgoing air duct and were constantly fanned by an air current moving at the rate of 0.88 miles per hour. Two boxes of apples of each variety were used under each storage condition. The Grimes were stored September 18, the Stayman Winesap October 12, and the Rome Beauty October 2. All were in the direct expansion storage room till October 21, when half of each lot was moved to the bunker system storage. The final notes on the Grimes were taken February 15 and on the Stayman Winesap and Rome Beauty March 25. All the apples were held at a temperature of 20° C. (68° F.) for four days before the notes were taken.

TABLE XI.—*Apple-scald in direct expansion and bunker systems of cold storage*

Kind of storage.	Percentage of scald.		
	Grimes.	Stayman Winesap.	Rome Beauty.
Direct expansion	35	12	11
Bunker	1	0	8

The results would indicate that the bunker system was much more favorable to scald prevention than the direct expansion. It should be noted, however, that with the bunker storage the apples were given one of the most favorable locations in the room so far as air circulation was concerned. Anemometer readings showed that the air in the lower corners of the room was practically stagnant and but little affected by the air circulation above. What the results in Table XI do show is that a continuous air circulation at the rate of 0.88 mile per hour practically eliminates scald on box apples.

In the experiment at Winchester, Va., all the apples were stored in large rooms cooled by a direct expansion system, but the different lots were variously located so as to receive different amounts of aeration. One of the storage rooms had two outside windows, each 3 feet wide and 5 feet high, in the west wall of the room, and two similar windows in the east wall. The doors in the elevator shaft were near the east end of the room. The windows and doors were thrown open on cool nights and the outside air admitted freely into the storage room. A $\frac{1}{10}$ horsepower ventilating fan was sometimes used in one of the doors. Such breezes as were obtained were so soon dissipated that it was never possible to obtain anemometer readings at a greater distance than 10 feet from any of the windows.

Because of the infrequency of cool nights in the fall of 1918 and the difficulty of having somebody at the storage rooms at the right time, only five ventilations were given during the critical period for scald. The first of these was made on November 12, and the others followed at weekly intervals. Considerable benefit was apparently derived from these ventilations, but probably not as much as from the daily fanning of the doors in connection with the regular storage-house operations. The apples of lot 1, Table XII, were stored in a corner of the room in the bottom of the stack, those of lot 2 near a west window in the middle of a large stack, and those of lot 3 in an aisle between an east window and the door into the elevator shaft. The apples of lot 4 were in an aisle near the door of a second storage room that was similar to the first but had no windows and received no special ventilations. The Stayman Winesap apples were stored September 25, and the final notes taken January 23; the Arkansas stored October 28, and the final notes taken January 30; and the York Imperial stored October 1 and October 29, and the final notes taken February 14 and March 8. Three barrels of each variety were used under each storage condition.

TABLE XII.—*Aeration in commercial cold storage*

Lot No.	Storage location.	Percentage of scald.					
		York Imperial.				Stayman Winesap.	Arkansas.
		Stored Oct. 1.		Stored Oct. 29.			
		Ventilated barrels.	Commercial barrels.	Ventilated barrels.	Commercial barrels.	Commercial barrels.	Commerical barrels.
1	In corner in bottom of stack	.....	80	.....	.....	.....	.....
2	Near window in middle of stack	15	44	.....	.....	49	.....
3	In aisle near window	12	32	1	10	32	28
4	In aisle of unventilated room	15	35	5	28	67	50

Of the first three lots of apples, all from the same room, those in the bottom of the stack at a distance from the windows and doors were scalded practically twice as much as any of the others; those surrounded by other barrels but near a window were next, while those in an aisle near a window had least scald. The apples of lot 4 which were in the aisle of the unventilated room were, in general, much worse scalded than those of lot 3, which were in the aisle of the ventilated room.

The results of the two experiments show a very close relationship between air circulation in cold-storage plants and scald prevention. It seems evident that the general management of the rooms and the arrangement of the stacks and the aisles are important factors in securing aeration of the fruit. The renewal of the air in the storage room is of

minor importance compared with sufficient stirring of the air within the room to enable the apples to throw off their waste gases.

STORAGE PACKAGES

In a question where the aeration of the fruit is involved, the nature of the package naturally plays an important part. In an earlier paper (5) preliminary experiments were reported covering a number of different temperatures and a great many different varieties of apples and showing that scald could always be produced by storing the apples in moist chambers and could always be prevented by storing them in open containers. Preliminary experiments were also reported showing that scald could be reduced in commercial storage by the use of ventilated barrels. The results reported in the following paragraph give confirmatory evidence of the importance of open packages in commercial storage.

The data reported in Table XIII were obtained in Wenatchee, Wash. The Grimes apples were picked September 18, were held in a laboratory until October 4, and were then placed in cellar storage. The lard cans remained closed until the apples were removed from storage. The final notes were taken February 14. The Rome Beauty apples were picked October 3, were held in cellar storage till November 2, and were then placed in cold storage. The lard cans remained closed till the apples were removed from storage. The final notes were taken February 14. The Stayman Winesap apples were picked October 12, were held in a cool, well-ventilated place till October 19, and then the lard cans were opened and all of the apples transferred to cold storage. The final notes were taken March 25. All lots were held in the open in a warm laboratory for three or more days before the final notes were taken. The following results were obtained on unwrapped apples, but similar contrasts were also obtained on wrapped apples.

TABLE XIII.—*Closed packages in storage*

Variety.	Percentage of scald.	
	In lard cans.	In commercial. boxes.
Grimes	42	24
Rome Beauty	68	25
Stayman Winesap	34	2

The results show that the tight package greatly increased the amount of scald.

The experiments reported in Table XIV were made in Winchester, Va., and Washington, D. C. The Grimes at Winchester were picked September 11, the Stayman Winesap September 25, the green York Imperial October 1, and the ripe York Imperial October 29. The Grimes at

Washington, D. C., were picked September 3, the Rome Beauty September 27, and the York Imperial October 10. The Grimes and Rome Beauty in the Washington experiment were from Franklin, Va., and the York Imperial from Greenwood, Va. The time of taking the final notes is given in the table. Three baskets and from 3 to 15 barrels of apples were used under each storage condition reported. The baskets held approximately a bushel of apples each and were of the low, tight form with overlapping slats. The ventilated barrels were made by cutting holes in the staves of the usual commercial barrels. Fifteen holes ¾ inch by 4 inches were made in each barrel, care being taken to have the openings well distributed and to avoid weakening the barrel by making cuts too near the bulge. A more satisfactory barrel can be obtained by having the cooper notch the staves before the barrel is made. The room in which the Winchester apples were stored received an occasional ventilation, while that in which the Washington apples were stored did not; but in both cases the apples were near the door.

TABLE XIV.—*Influence of baskets and ventilated barrels upon apple-scald*

Treatment.	Percentage of scald.						
	Ventilated room, Winchester, Va.				Unventilated room, Washington, D. C.		
	Grimes, Jan. 11.	Stayman Winesap, Jan. 23.	York Imperial.		Grimes, Dec. 20.	York Imperial, Jan. 23.	Rome Beauty, Jan. 28.
			Green, Feb. 14.	Mature, Feb. 20.			
Immediate storage:							
Commercial barrels	33	49	45	25	35	20	28
Ventilated barrels	8	16	15	1	30	8	20
Baskets	.....	18	15	.....	.....	.....	.....
Delayed storage:							
Commercial barrels	29	76	65	.....	12	60	80
Ventilated barrels	20	.....	35	.....	3	17	0
Baskets	.....	25	45	.....	.....	.....	.....

The results with the baskets were similar to those with the ventilated barrels. With the immediate storage in the ventilated room, the ventilated barrels in every case reduced the scald to at least one-third of that in the commercial barrels; but with the fruit in the unventilated room, the ventilated barrels caused only a slight reduction in scald. In the delayed storage, the ventilated barrels resulted in a great decrease in scald in nearly every case, often reducing the percentage of the disease below that in the immediately stored commercial barrel. The results, as a whole, show that the ventilated barrel can be used to great economic advantage in the prevention of apple-scald.

The better aeration of the ventilated barrels was evidenced in the quicker rate of cooling on going into storage and in the composition of

the air of the barrels as well as in the prevention of apple-scald. These facts are brought out in a graphic manner in figures 1 and 2.

In the tests on rate of cooling, the temperature records were obtained by means of resistance thermometers, the thermometer bulbs being forced into apples in the middle of the barrels and the readings taken from the outside with an indicator without disturbing the fruit.

It will be noted from the curves in figure 1 that during the first few days in storage the apples in the ventilated barrels were from 5° to 10° F.

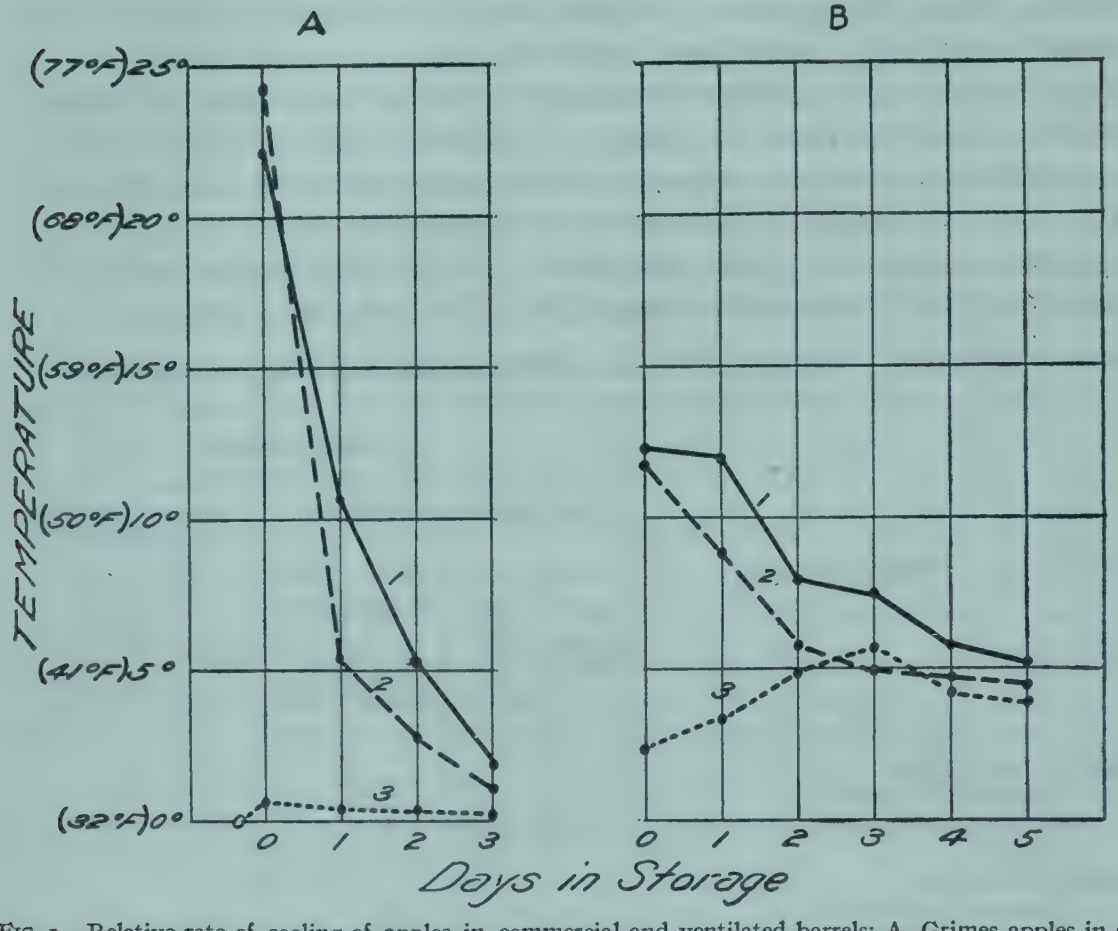

FIG. 1.—Relative rate of cooling of apples in commercial and ventilated barrels: A, Grimes apples in a storage room already filled with cold fruit; B, York Imperial apples in a storage room still receiving a large bulk of warm fruit. Curve 1 shows the temperature of the fruit in the center of a commercial barrel; curve 2, the temperature of the fruit in the center of a ventilated barrel; and curve 3, the temperature of the storage room.

colder than those in the commercial barrels. The quicker cooling secured by the ventilated barrels has a value in itself; but since the cooling is accomplished by air currents, the temperature contrast is also of interest as proof of the much freer exchange of air allowed by the more open barrels.

The relative carbon-dioxid content of the air in the ventilated and commercial barrels during the first weeks of storage is shown in figure 2. The gas analyses in figure 2, A, were made with the Pettersson gas apparatus and those in figure 2, B, with the Allen-Moyer Orsat apparatus. The samples were taken from the center of the barrel, small tubes having been arranged for this purpose at the time the apples were packed. A study

of the curves shows that there was usually more than twice as much carbon dioxid in the air of the commercial barrels as in the air of the ventilated barrels and but little more in the air of the ventilated barrels than in the air of the storage room. As was pointed out earlier in the paper (p. 213) small quantities of carbon dioxid do not appear to be harmful to apples; but since the fruit is continually giving off this gas, the quantity of it in the storage air does serve as an indicator of the extent of ventilation. The results give further evidence of a decided contrast between the aeration secured in the ventilated and commercial barrels.

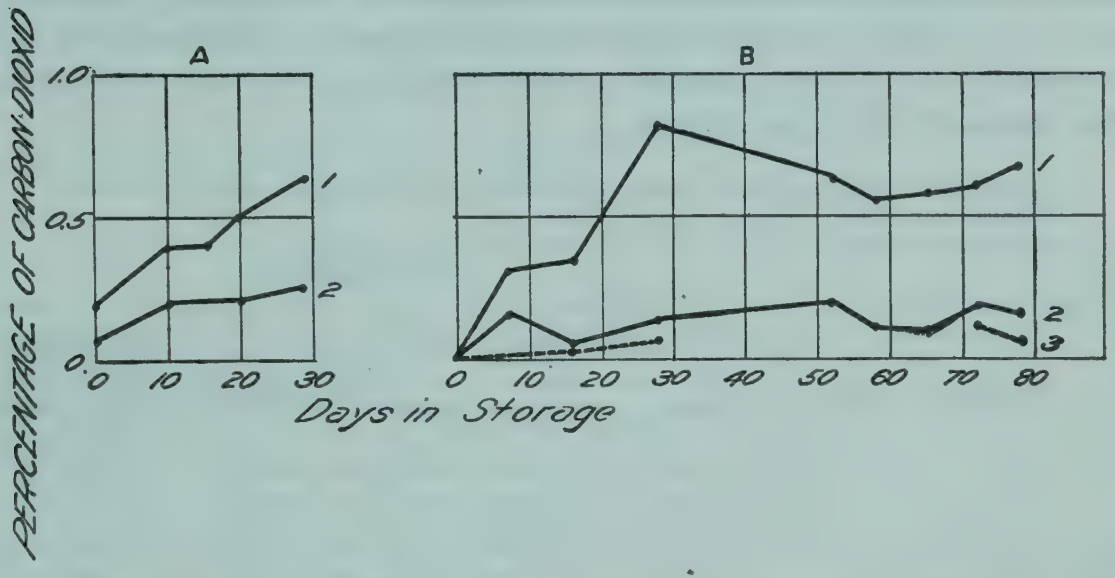

FIG. 2.—Relative carbon-dioxid content of the air in ventilated and commercial barrels during the first weeks of storage: A, Grimes apples in a storage room already filled with cooled fruit; B, York Imperial apples in a storage room still receiving a large bulk of fresh fruit. Curve 1 shows the percentage of carbon dioxid in the air of a commercial barrel; curve 2, the percentage in the air of a ventilated barrel; and curve 3, the percentage in the air of the storage room.

WAXES, FATS, OILS, AND OTHER GAS ABSORBENTS AS AGENCIES IN SCALD PREVENTION

Preliminary experiments were reported in an earlier paper (5) indicating that certain waxes and oils could be used as absorbents for the gases that are instrumental in producing apple-scald. In the following experiments the earlier results are confirmed and the list of gas absorbents greatly extended.

The neutral mineral oil wrappers were obtained from an oiled manila paper similar to that used in meat markets. The paraffin wrappers A were made by saturating ordinary apple wrappers with paraffin; the paraffin wrappers B and D were made from very light-weight commercial paraffin paper; and the paraffin wrappers C from a fairly heavy commercial paraffin paper. The glassine wrappers were from paper sold commercially under that name and apparently contained no wax or oil. All the other wrappers reported in Tables XV, XVI, and XVII were prepared by saturating the usual commercial apple wrappers with the given oil or wax.

The results reported in Table XV were obtained in experimental storage boxes, the separate lots of apples being held in moist chambers. Only 10 apples were used in each lot, but great care was taken that the different lots should be as nearly alike as possible. The tests were carried out under several different conditions with remarkably consistent results. The Grimes of August 21 were quite green and were placed under the given storage conditions the day after picking. The Grimes of October 29 were picked September 4, packed in barrels, and held in commercial cold storage from September 5 to October 28. The Rhode Island Greening apples were held in barrels in cold storage till November 5, and the Yellow Newtown apples till December 19. The wrapper experiments on the last three lots were started at the time the apples were removed from cold storage.

TABLE XV.—*Effect of oils, waxes, and other gas absorbents upon apple-scald*

Wrappers or packing.	Percentage of scald.								
	Grimes, Aug. 21, 1918.				Grimes, Oct. 29, 1918, after 14 weeks at—			Rhode Island Greening, after 10 weeks at 15° C.	Yellow Newtown after 14 weeks at 5° C.
	After 10 weeks at—		After 16 weeks at—						
	15° C.	10° C.	2½° C.	0° C.	5° C.	2½° C.	0° C.		
None, control	60	40	43	58	90	52	53	85	40
Wrappers, commercial	45	.....	38	50	63	55	70	41	35
Wrappers, paraffin A	12	.....	.....	20	60	25	35	.....	6
Wrappers, paraffin B	12	.....	15	30	.....	.....	.....	.....	.....
Wrappers, paraffin C	15	.....	32	38	.....	.....	.....	.....	.....
Wrappers, glassine	.....	.....	.....	.....	.....	.....	.....	70	50
Wrappers beeswax 30 per cent, vaseline 70 per cent	0	.....	3	30	3	10	3	.....	.....
Wrappers, beeswax 30 per cent, olive oil 70 per cent	1	.....	32	23	.....	.....	.....	.....	0
Wrappers, cacao butter 70 per cent, vaseline 30 per cent	1	.....	20	18	.....	.....	.....	.....	.....
Wrappers, cacao butter 70 per cent, olive oil 30 per cent	.....	.....	12	18	.....	.....	.....	.....	.....
Wrappers, olive oil	.....	.....	.....	.....	.....	.....	.....	.....	0
Wrappers, neutral mineral oil	0	.....	5	9	.....	.....	.....	.....	0
Wrappers, vaseline	0	.....	18	18	.....	.....	.....	0	0
Wrappers, cacao butter	3	.....	20	35	.....	.....	.....	0	.....
Wrappers, beeswax	.....	.....	.....	.....	.....	.....	.....	9	0
Wrappers, ceresin wax	.....	.....	.....	.....	.....	.....	.....	.....	0
Wrappers, Japanese wax	.....	.....	.....	.....	.....	.....	.....	.....	0
Wrappers, Carnauba wax	.....	.....	.....	.....	.....	.....	.....	.....	10
Wrappers, apple wax	.....	.....	.....	.....	.....	.....	.....	.....	0
Wrappers, ivory soap	.....	.....	.....	.....	.....	.....	.....	.....	10
Wrappers, glycerin	.....	.....	.....	.....	.....	.....	.....	.....	30
Wrappers, sugar, 100 per cent solution	.....	.....	.....	.....	.....	.....	.....	.....	30
Wrappers, lard	.....	.....	.....	.....	.....	.....	5	17	0
Wrappers, tallow	.....	.....	.....	.....	.....	.....	7	0	0
Wrappers, cow butter (unsalted)	.....	.....	.....	.....	.....	.....	4	0	0
Wrappers, linseed oil	.....	.....	.....	.....	.....	.....	.....	30	.....
Wrappers, neat's-foot oil	.....	.....	.....	.....	.....	.....	.....	0	.....
Wrappers, cottonseed oil	.....	.....	.....	.....	.....	.....	.....	40	.....
Wrappers, corn oil	.....	.....	.....	.....	.....	.....	.....	20	.....
Wrappers, peanut oil	.....	.....	.....	.....	.....	.....	.....	.....	0
Wrappers, castor oil	.....	.....	.....	.....	.....	.....	.....	.....	0
Charcoal, animal	15	35	.....	.....	.....	.....	.....	.....	.....
Charcoal, wood	30	35	.....	.....	.....	.....	.....	.....	.....
Charcoal, norite	60	30	.....	.....	.....	.....	.....	.....	.....
Cork, granulated, wet	7	40	.....	.....	.....	.....	.....	.....	.....
Cork, granulated, dry	1	.....	.....	.....	.....	.....	.....	.....	.....
Excelsior	30	.....	.....	.....	.....	.....	.....	.....	.....
Brick, granulated	30	.....	.....	.....	.....	.....	.....	.....	.....
Pumice stone, granulated	45	15	.....	.....	.....	.....	.....	.....	.....
Oatmeal	5	20	.....	.....	.....	.....	.....	.....	.....

Linseed oil and castor oil injured the skin of the apples wherever the wrapper was in close contact with the fruit.

The tests reported in Table XVI were made in a commercial cold-storage plant at Wenatchee, Wash. The apples were packed in boxes, one layer of apples being used for each treatment with a thick layer of newspapers between lots. The Grimes apples were stored September 18, were removed to a temperature of 20° C. (68° F.) on February 6, and the final notes taken February 15. The Stayman Winesap apples were stored October 12 and the Rome Beauty October 10. The Stayman Winesap apples were in storage two weeks before the fruit was wrapped. The apples of both these varieties were removed to a temperature of 20° C. (68° F.) on March 15 and the final notes taken March 24.

TABLE XVI.—*Box apples wrapped and unwrapped*

Kind of wrapper.	Percentage of scald.		
	Grimes.	Rome Beauty.	Stayman Winesap.
None, control	75	12	16
Commercial, no wax or oil	60	.....	19
Paraffin B	.....	.....	11
Paraffin C	.....	.....	17
Paraffin D	.....	6	.....
Glassine	.....	.....	47
Paraffin 50 per cent, vaselin 50 per cent	0	1	.....
Vaseline	2	0	.....
Beeswax 30 per cent, vaseline 70 per cent	0	0	.....
Cacao butter 70 per cent, vaseline 30 per cent	5	0	.....
Beeswax 30 per cent, olive oil 70 per cent	0	3	.....
Cacao butter 75 per cent, olive oil 25 per cent	0	1	.....
Cacao butter	0	.....	.....
Mineral oil	.....	.....	0

The tests reported in Table XVII were made with eastern barreled apples. Only about one-third of the apples of the barrel were wrapped. The barrels were filled approximately half full of unwrapped apples, a bushel of wrapped apples added, and the remainder of the barrel filled with unwrapped apples. The Grimes were from Vienna, Va., and were placed in commercial cold storage at Washington, D. C., on September 18. The York Imperial were from Winchester, Va., and were placed in commercial cold storage at that point on October 1. The apple-scald notes on the Grimes were taken December 20 and those on the York Imperial March 12. Both lots were held at a temperature of 20° C. (68° F.) for a period of three days before the notes were taken. See Table XVII.

The results in Tables XV, XVI, and XVII give conclusive proof that there is a wide range of materials that are capable of absorbing the harmful substances produced in apple storage. With some of the materials, such as oatmeal and granulated cork, a part of the good

effects might possibly be attributed to the influence upon humidity; but the results as a whole require a different explanation. As was pointed out earlier in the paper (p. 216) it seems practically certain that the beneficial effects, particularly of the waxes, fats, and oils, are due to their power of absorbing esters or other similar products thrown off in gaseous form by the apple.

TABLE XVII.—*Apple wrappers in commercial barrel storage*

Kind of wrapper.	Percentage of scald.					
	Grimes.			York Imperial.		
	Wrapped.	Not wrapped.	Next layer to wrapped apples.	Wrapped.	Not wrapped.	Next layer to wrapped apples.
None, control barrels	.....	38	.....	.....	40	.....
Commercial, no wax or oil	27	.....	30	28	.....	30
Paraffin A	18	.....	33	35	.....	30
Paraffin B	15	.....	23	10	.....	30
Paraffin C	23	.....	32	20	.....	.....
Beeswax 30 per cent, vaselin 70 per cent	9	.....	23	2	.....	35
Beeswax 30 per cent, oilve oil 70 per cent	18	.....	33	5	.....	18
Cacoa butter 70 per cent, olive oil 30 per cent	8	.....	32	1	.....	12
Mineral oil	0	.....	18	.....	.....	.....

The results furnish some very interesting contrasts. In most tests the commercial wrappers caused little or no reduction in scald, and the paraffin wrappers were but little better, while nearly all the other wrappers caused a decided decrease in the disease. Particularly good results were obtained with fats like cow butter and tallow and with neat's foot oil and mineral oils. It should be noted that there is a close correlation between the ability of the various substances to control apple-scald and their capacity for absorbing gases.

A very significant point is brought out in Table XVII in the extension of the scald reduction to the apples that were adjacent to the wrapped ones. With some of the more efficient wrappers the scald on the contiguous fruit was reduced to less than one-half of that on the fruit in other barrels or in the distant parts of the same barrel. In most cases this effect extended only to apples actually in contact with the wrapped apples, but with the olive and mineral oil wrappers there was an evident decrease in scald at a distance of several layers from the wrapped fruit. These results can hardly be explained by any other theory than that the good effects of the wrappers are largely due to the gas-absorbing capacity of the fats and oils they contain.

PRACTICAL CONSIDERATION

In recording the percentages of scald in the foregoing experiments, consideration has been given to both the number of apples affected and the intensity of the disease; the scald ratings therefore bear a very close relation to the actual damage done to the fruit and to the reduction in price resulting from it. In an average market, the loss in price on the apples would be about half that of the percentage of scald recorded—for example, apples that have been marked as having 80 per cent of scald would ordinarly be sold at a reduction of about 40 per cent in price, apples having 50 per cent of scald at a reduction of 25 per cent, and apples having 5 or 10 per cent of scald at little or no reduction. It is possible, therefore, to obtain a fairly close estimate of the effect of the various treatments upon the value of the fruit. It will be seen by reference to Table XII that barreled apples stored in the bottom of the stack at a distance from the window were damaged by scald to the extent of 40 per cent of their value (80 per cent of scald), while similar apples near the window or in the aisles were damaged but 15 per cent, and the apples in the ventilated barrels but 6 per cent—an amount that might be entirely overlooked in many markets. In the wrapper experiments as shown in Tables XV, XVI, and XVII, the unwrapped apples and those in commercial wrappers were damaged by scald to the extent of from 20 to 40 per cent of their value while those wrapped in the best of the waxed papers were practically free from injury.

These estimates of damage are based on the assumption that the fruit becomes warm before being used. If the apples were sold on a northern market in cold weather, the loss from scald might not be felt by the dealer but be largely passed along to the consumer; but if it were necessary to expose the fruit in moderately warm weather the loss would be shown in the actual selling price. Whether the scald damage becomes evident on the market or only after the fruit has passed to the hands of the consumer, the loss is a real one. Apples that should have remained in good condition for several weeks under common storage conditions are rendered unfit for anything but immediate consumption and even undesirable for that. Not only does the scalded condition gradually spread to considerable depth in the tissue of the apple but the death of the skin exposes the softer tissues beneath to the action of blue mold (*Penicillium expansum*) and other rot organisms, and rapid decay follows. Apples with a sound epidermis are practically immune to rot at high as well as low temperatures (*3*), but apples with the skin killed by scald are doomed to early destruction.

In the apple trade the time at which scald appears on the fruit receives more consideration than the severity of the disease when it occurs. Anything that will postpone the development of apple-scald means a

greater freedom in marketing and fewer rush sales. In the experiments that have been reported no statements have been made as to the time when scald first appeared on the different lots of fruit, but a record was kept of this whenever possible. In the experiment reported in Table XII the York Imperial apples in commercial barrels in the aisle had 32 per cent of scald on January 28, and those in ventilated barrels 10 per cent of scald. On March 12, approximately 6 weeks later, the apples in the ventilated barrels had increased in scald, but only to 18 per cent, and, as is shown in Table XVII, the apples of this same lot that were in the best grade of wrappers were still entirely free from scald. It was impossible to obtain an early record of the apples in commercial barrels in the bottom of the stack (Table XII); but judging from the usual rate of development and the fact that they had 80 per cent of scald on January 28 it is probable that they had 20 to 30 per cent of scald by January 1. In other words, York Imperial apples in commercial barrels in the bottom of the stack were scalded badly enough to have their market value affected by January 1; similar apples in the aisle did not reach the same degree of scald till 4 weeks later; those in ventilated barrels in the aisle had scarcely reached it at the end of 10 weeks; and apples in waxed wrappers were entirely free from scald at the end of this period. What this means to the trade can be readily seen. On the one hand, apples must either be sold so early as to be out of season, or else disposed of for immediate consumption at a later date; on the other hand, if the fruit receives sufficient aeration in storage or is protected by oiled wrappers the dealer may choose his own time for selling and can expose his fruit on the market or ship it to distant points without fear of its going down with scald.

A study of the market products as they pass to the consumer will convince anyone of the enormous food and money losses resulting from apple-scald. It is the opinion of the writers that with the present method of handling apples the losses from this disease are greater than those from all other transportation and storage diseases of the apple, but in spite of all this direct loss it seems to them that the greatest injury to the apple trade comes from the effects of scald upon public confidence. A dealer or consumer buys with the assurance that the apples are of high quality and in good condition, and the seller may really believe them to be; but if the fruit becomes somewhat warmer before the buyer has an opportunity to inspect it he finds a scalded, rotten-looking lot of apples and naturally concludes that he has been cheated. At the present time there is much discussion as to the best methods of increasing apple consumption by increasing exports to foreign countries, extending the trade in the South, and increasing the shipments to small cities that can not handle car load lots. Apple-scald is one of the great barriers to this trade expansion, the disease not only often making it impossible to deliver

fruit in good condition but serving also as a continual source of misunderstanding.

The actaul losses caused by scald and the uncertainty it introduces into the apple trade add greatly to the cost of market operation and help to widen the gap between the producer's and the consumer's prices. The foregoing experiments show that it is a preventable disease and that with proper methods of handling the apples in the orchard and in transportation and storage the disease can be reduced to a negligible quantity if not entirely eliminated.

SUMMARY

(1) The foregoing experiments show that the occurrence of apple-scald is determined by orchard, packing house, transportation, and storage conditions.

(2) As has been shown by other investigators, mature fruit has in general scalded less than immature; but it has also been found that the fruit surfaces just changing from green to yellow have scalded worse than those that were a leaf green and worse than those that had more completely changed to yellow. Well-colored red fruit surfaces have been practically immune to scald.

(3) Apples from trees receiving heavy irrigation have scalded worse than those from trees receiving light irrigation. This was found not to be due to the greater number of large apples in the former case but to some forcing effect that increased the susceptibility to scald in both large and small apples.

(4) Delayed storage has increased or decreased apple-scald, depending upon the amount of aeration the apples received during delay.

(5) Apples in ventilated barrels have developed less than one-third as much scald as those in commercial barrels when both were held in a storage room that received an occasional ventilation, but where the storage room received little or no ventilation the ventilated barrels caused but little decrease in scald.

(6) The amount of scald developed in cold-storage plants has varied greatly with the location in the room. Apples near the aisle or near a door have scalded far less than those in the bottom of the stack. Boxed apples exposed to a continuous air current of 0.88 mile per hour in a commercial storage plant have been practically free from scald, while similar apples that did not receive the constant fanning became badly scalded. Stirring of the storage air has been found more important than its renewal in the prevention of apple-scald.

(7) The ordinary commercial apple wrappers have caused but little decrease in scald, and paraffin wrappers have been but slightly better, but wrappers impregnated with various fats and oils have either entirely prevented the disease or reduced it to a negligible quantity. In barrel

experiments in which only part of the fruit was wrapped, scald has been greatly reduced on the apples adjacent to the wrapped ones as well as on the wrapped apples themselves.

(8) Typical scald has been artificially produced in a few days' time by exposing apples to the vapors of ethyl acelate, amyl acetate, or methyl butyrate.

(9) The manner in which scald can be produced artificially and the different methods of control indicate that the disease is due to the accumulation of esters or similar products of the apple in the tissues of the fruit and in the surrounding air. The vapors of these substances can be carried away by air currents or absorbed by fats and oils.

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NITROGEN METABOLISM OF TWO-YEAR-OLD STEERS

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PLAN OF THE EXPERIMENT

The present paper is one of several reports on a digestion and metabolism experiment conducted at the Illinois Agricultural Experiment Station upon eight 2-year-old steers.[1] This paper reports the nitrogen balance of each steer as determined for each week during a total period of 37 weeks. The experiment was divided into five experimental periods. During the first period the ration consisted of clover hay and ground corn in equal amounts by weight; during the second, of one part of clover hay and three parts of ground corn; during the third, of one part of clover hay and five parts of ground corn; and during the fourth and fifth, of one part of clover hay, four parts of ground corn, and one part of linseed oil meal. The last proportion was maintained to the end of the experiment. These changes are comparable to the changes often made in the proportions of roughage and concentrates in ordinary feeding practice. The first experimental period was five weeks in length; the second, third, and fourth were each six weeks in length; and the fifth was four weeks in length. The changes in the ration made from one test period to another were effected very gradually in transitional periods, one of which immediately followed each experimental period. The first and third transitional periods were two weeks in length; the second and fourth were three weeks in length. Table I gives the experimental periods and the feeds given in each period.

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TABLE I.—*Ratios of hay, corn, and linseed meal in rations at different periods of the experiment*

Period No.	Weeks included in experiment.	Number of weeks in period.	Ratio of hay to corn to linseed meal.
1	1–5	5	1:1:0
2	8–13	6	1:3:0
3	17–22	6	1:5:0
4	25–30	6	1:4:1
5	34–37	4	1:4:1

To determine the effect of variations in the amount of feed consumed the eight steers were divided into four lots of two animals each, and each lot was given throughout the experiment an amount of feed different from that received by the other lots. The lots were as similar as possible in age, condition, and breeding. One lot was given just enough feed to maintain the weights of the steers about constant; another, as much as the steers would eat readily; another, an amount of feed equal to the maintenance ration plus one-third of the difference between the maintenance and the full feed rations; and another, an amount equal to the maintenance ration plus two-thirds of the difference between the maintenance and full feed rations. Beginning with the thirty-first week, one steer each from the maintenance, the one-third, and the two-thirds feed lots was gradually put on a full feed ration and kept upon it until the end of the experiment.

Tables II and III show the consumption of digestible crude protein and net energy per period. Table IV gives the weights of the steers. Table V gives the amounts of nitrogen consumed, the amounts of urinary nitrogen, fecal nitrogen, total excretory nitrogen, and the nitrogen balance of each steer per week.

TABLE II.—*Digestible crude protein consumed daily per 1,000 pounds live weight*

[Results expressed in pounds]

Period No.	Weeks included in experiment.	Ratio of hay to corn to oil-meal.	Maintenance lot.		One-third feed lot.		Two-thirds feed lot.		Full feed lot.	
			Steer 650.	Steer 656.	Steer 666.	Steer 669.	Steer 652.[a]	Steer 665.	Steer 663.[b]	Steer 661.
1	1–5	1:1:0	0.56	0.65	0.82	0.74	0.88	0.90	1.04	0.96
2	8–13	1:3:0	.44	.45	.67	.69	.81	.87	.85	.85
3	17–22	1:5:0	.40	.40	.54	.56	.60	.67	.66	.65
4	25–30	1:4:1	.83	.82	.99	1.00	1.12	1.20	1.19	1.39
5[c]	34–37	1:4:1	1.47	.77	1.34	.98	1.31	1.15	.....	1.34

[a] Removed at end of thirty-fourth week.
[b] Removed at end of thirtieth week.
[c] Steers 650, 666, and 652 were on full feed in period 5.

TABLE III.—*Net energy consumed daily per 1,000 pounds live weight* [a]

[Results expressed in therms]

Period No.	Weeks included in experiment.	Ratio of hay to corn to oilmeal.	Maintenance lot.		One-third feed lot.		Two-thirds feed lot.		Full feed lot.	
			Steer 650.	Steer 656.	Steer 666.	Steer 669.	Steer 652.[b]	Steer 665.	Steer 663.[c]	Steer 661.
1.....	1–5	1:1:0	6.98	7.13	9.46	8.64	11.05	11.17	12.69	12.43
2.....	8–13	1:3:0	6.95	7.00	9.80	9.77	12.25	12.59	13.67	13.20
3.....	17–22	1:5:0	6.73	6.73	8.87	9.35	11.16	11.55	11.53	10.75
4.....	25–30	1:4:1	6.59	6.47	8.37	8.41	9.90	10.86	10.52	12.22
5[d].....	34–37	1:4:1	12.28	6.03	11.49	6.74	11.27	9.88	.....	11.96

[a] Assuming that the energy requirements vary directly as the two-thirds power of the live weight.
[b] Removed at end of thirty-fourth week.
[c] Removed at end of thirtieth week.
[d] Steers 650, 666, and 652 were on full feed in period 5.

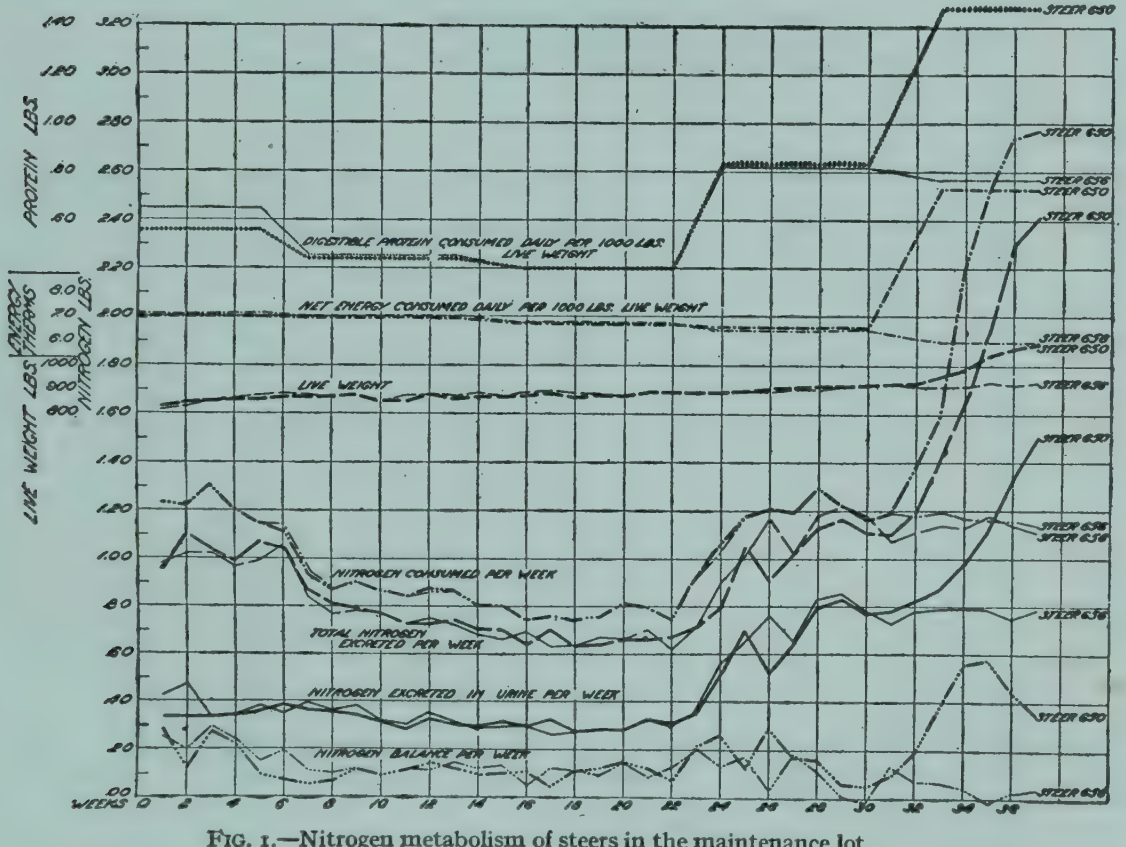

FIG. 1.—Nitrogen metabolism of steers in the maintenance lot.

RELATION OF NITROGEN CONSUMED TO NITROGEN EXCRETED

MAINTENANCE LOT.—Figure 1 shows the daily consumption of digestible crude protein per 1,000 pounds live weight during each period, the daily consumption of net energy per 1,000 pounds live weight during each period, the average live weight per week, the nitrogen consumed per week, the total nitrogen excreted per week, the urinary nitrogen per week, and the nitrogen balance per week of the two steers of the maintenance lot.

TABLE IV.—*Weights of steers at beginning and end of each period*

[Results expressed in pounds]

Period No.	Week on which test ended.	Ratio of hay to corn to oilmeal.	Maintenance lot.		One-third feed lot.		Two-thirds feed lot.		Full feed lot.	
			Steer 650.	Steer 656.	Steer 666.	Steer 669.	Steer 652.[a]	Steer 665.	Steer 663.[b]	Steer 661.
1.....	1	1:1:0	814	809	849	901	869	886	873	1,004
1.....	5	1:1:0	869	878	937	970	975	1,004	992	1,093
2.....	8	1:3:0	864	869	932	978	977	1,018	1,019	1,109
2.....	13	1:3:0	859	872	988	1,033	1,071	1,084	1,109	1,191
3.....	17	1:5:0	879	882	1,000	1,053	1,098	1,143	1,113	1,217
3.....	22	1:5:0	881	886	1,023	1,107	1,161	1,186	1,202	1,255
4.....	25	1:4:1	895	891	1,027	1,115	1,177	1,204	1,244	1,295
4.....	30	1:4:1	930	922	1,084	1,178	1,250	1,283	1,320	1,404
5[c]	34	1:4:1	972	912	1,138	1,195	1,275	1,309	.....	1,462
5.....	37	1:4:1	1,087	934	1,197	1,220	(1,286)	1,348	.....	1,518

[a] Removed at end of thirty-fourth week.
[b] Removed at end of thirtieth week.
[c] Steers 650, 667, 666, and 652 were on full feed in period 5.

TABLE V.—*Nitrogen metabolism of steers*

MAINTENANCE LOT

[Expressed as pounds per period of seven days]

Week No.	Steer 650.					Steer 656.				
	Nitrogen ingested.	Nitrogen excreted.			Balance.	Nitrogen ingested.	Nitrogen excreted.			Balance.
		In urine.	In feces.	In urine and feces.			In urine.	In feces.	In urine and feces.	
1	1.231	0.329	0.620	0.949	0.282	1.231	0.425	0.559	0.984	0.247
2	1.217	.327	.771	1.098	.119	1.217	.468	.550	1.018	.199
3	1.304	.328	.708	1.036	.268	1.304	.328	.688	1.016	.288
4	1.203	.340	.646	.986	.217	1.203	.343	.622	.965	.238
5	1.145	.355	.703	1.058	.087	1.145	.382	.611	.993	.152
6	1.114	.378	.666	1.044	.070	1.142	.350	.597	.947	.195
7	.926	.361	.513	.874	.052	.947	.391	.446	.837	.110
8	.874	.353	.460	.813	.061	.874	.364	.408	.772	.102
9	.896	.341	.445	.786	.110	.896	.376	.405	.781	.115
10	.861	.310	.460	.770	.091	.861	.311	.459	.770	.091
11	.843	.279	.453	.732	.111	.843	.297	.431	.728	.115
12	.867	.321	.404	.725	.142	.860	.346	.405	.751	.109
13	.861	.296	.445	.741	.120	.861	.310	.409	.719	.142
14	.796	.294	.411	.705	.091	.796	.294	.383	.677	.119
15	.797	.308	.392	.700	.097	.797	.288	.357	.663	.134
16	.744	.286	.357	.643	.101	.744	.289	.403	.692	.052
17	.755	.316	.387	.703	.052	.755	.259	.375	.634	.121
18	.740	.266	.369	.635	.105	.740	.267	.364	.631	.109
19	.763	.276	.368	.644	.119	.763	.277	.391	.668	.095
20	.808	.276	.387	.663	.145	.808	.276	.389	.665	.143
21	.787	.319	.344	.663	.124	.787	.325	.377	.702	.085
22	.742	.314	.360	.674	.068	.742	.301	.316	.617	.125
23	.917	.353	.355	.708	.209	.917	.358	.355	.713	.204
24	1.056	.512	.283	.795	.261	1.027	.564	.332	.896	.131
25	1.175	.691	.358	1.049	.126	1.175	.651	.356	1.007	.168
26	1.205	.521	.393	.914	.291	1.205	.758	.411	1.169	.036
27	1.194	.640	.383	1.023	.171	1.194	.648	.370	1.018	.176
28	1.293	.795	.338	1.133	.160	1.293	.830	.348	1.178	.115
29	1.223	.834	.331	1.165	.058	1.223	.854	.364	1.218	.005
30	1.161	.767	.346	1.113	.048	1.161	.778	.401	1.179	—.018
31	1.194	.783	.317	1.100	.094	1.194	.732	.335	1.067	.127
32	1.385	.819	.376	1.195	.190	1.181	.781	.333	1.114	.067
33	1.792	.866	.549	1.415	.377	1.200	.792	.352	1.144	.056
34	2.200	.979	.666	1.645	.555	1.171	.787	.343	1.130	.041
35	2.526	1.132	.818	1.950	.576	1.158	.785	.395	1.180	—.022
36	2.742	1.340	.963	2.303	.439	1.162	.749	.389	1.138	.024
37	2.768	1.512	.903	2.415	.353	1.142	.793	.314	1.107	.035

TABLE V.—*Nitrogen metabolism of steers*—Continued

ONE-THIRD FEED LOT

[Expressed as pounds per period of seven days]

Week No.	Steer 666.					Steer 669.				
	Nitrogen ingested.	Nitrogen excreted.			Balance.	Nitrogen ingested.	Nitrogen excreted.			Balance.
		In urine.	In feces.	In urine and feces.			In urine.	In feces.	In urine and feces.	
1	1.705	0.334	0.603	0.940	0.765	1.761	0.431	0.902	1.333	0.428
2	1.686	.427	.915	1.342	.344	1.741	.479	1.122	1.601	.140
3	1.806	.443	.962	1.405	.401	1.865	.479	.930	1.409	.456
4	1.722	.448	.962	1.410	.312	1.750	.506	.936	1.442	.308
5	1.626	.504	.974	1.478	.148	1.677	.566	1.013	1.579	.098
6	1.613	.482	.962	1.444	.169	1.714	.534	.823	1.357	.357
7	1.409	.553	.798	1.351	.058	1.481	.585	.801	1.386	.095
8	1.349	.537	.670	1.207	.142	1.406	.564	.727	1.291	.115
9	1.404	.542	.679	1.221	.183	1.462	.555	.675	1.230	.232
10	1.385	.486	.674	1.160	.225	1.439	.545	.660	1.205	.234
11	1.376	.579	.681	1.260	.116	1.421	.564	.660	1.224	.197
12	1.415	.571	.663	1.234	.181	1.480	.526	.654	1.180	.300
13	1.405	.564	.660	1.224	.181	1.480	.564	.653	1.217	.263
14	1.251	.563	.625	1.188	.063	1.371	.573	.679	1.246	.125
15	1.241	.510	.595	1.105	.136	1.374	.600	.579	1.179	.195
16	1.160	.469	.536	1.005	.155	1.287	.591	.565	1.156	.131
17	1.165	.474	.587	1.061	.104	1.294	.550	.573	1.123	.171
18	1.120	.500	.555	1.055	.065	1.226	.549	.587	1.136	.090
19	1.156	.457	.544	1.001	.155	1.243	.531	.589	1.120	.123
20	1.224	.396	.547	.943	.281	1.316	.570	.531	1.101	.215
21	1.192	.586	.546	1.132	.060	1.282	.559	.605	1.164	.118
22	1.060	.456	.489	.945	.115	1.145	.554	.506	1.060	.085
23	1.317	.591	.470	1.061	.256	1.425	.652	.565	1.217	.208
24	1.537	.538	.555	1.093	.444	1.659	.779	.631	1.410	.249
25	1.679	.540	.530	1.070	.609	1.813	1.125	.624	1.749	.064
26	1.721	.602	.598	1.200	.521	1.859	.994	.668	1.662	.197
27	1.706	.915	.631	1.546	.160	1.843	1.080	.620	1.700	.143
28	1.847	1.001	.510	1.511	.336	1.995	1.168	.577	1.745	.250
29	1.799	1.069	.652	1.721	.078	1.943	1.215	.576	1.791	.152
30	1.707	1.159	.563	1.722	—.015	1.843	1.219	.592	1.811	.032
31	1.773	1.151	.553	1.704	.069	1.954	1.195	.595	1.790	.164
32	1.958	1.137	.558	1.695	.263	1.932	1.225	.592	1.817	.115
33	2.374	1.284	.772	2.056	.318	1.964	1.210	.558	1.768	.196
34	2.625	1.418	.887	2.305	.320	1.916	1.225	.588	1.813	.103
35	2.741	1.603	.981	2.584	.157	1.894	1.208	.584	1.792	.102
36	2.863	1.682	1.043	2.725	.138	1.902	1.199	.573	1.772	.130
37	2.872	1.681	1.119	2.800	.072	1.868	1.224	.519	1.743	.125

TABLE V.—*Nitrogen metabolism of steers*—Continued

TWO-THIRDS FEED LOT

[Expressed as pounds per period of seven days]

Week No.	Steer 652.[a]					Steer 665.				
	Nitrogen ingested.	Nitrogen excreted.			Balance.	Nitrogen ingested.	Nitrogen excreted.			Balance.
		In urine.	In feces.	In urine and feces.			In urine.	In feces.	In urine and feces.	
1	2.202	0.438	1.276	1.714	0.488	2.286	0.454	1.340	1.794	0.492
2	2.180	.571	1.344	1.915	.265	2.265	.521	1.366	1.887	.378
3	2.336	.488	1.324	1.812	.524	2.429	.584	1.201	1.785	.644
4	2.212	.557	1.227	1.784	.428	2.298	.566	1.373	1.929	.359
5	2.114	.599	1.273	1.872	.242	2.209	.628	1.399	2.027	.182
6	2.116	.609	1.168	1.777	.339	2.268	.623	1.362	1.985	.283
7	1.896	.655	1.042	1.697	.199	1.881	.639	1.148	1.787	.094
8	1.824	.659	1.004	1.663	.161	1.934	.621	.958	1.579	.355
9	1.911	.680	.945	1.625	.286	2.028	.644	1.012	1.656	.372
10	1.906	.597	.962	1.559	.347	2.019	.724	1.007	1.731	.288
11	1.894	.667	.987	1.654	.240	2.013	.670	1.047	1.717	.296
12	1.948	.616	.939	1.555	.393	2.096	.686	.974	1.660	.436
13	1.934	.629	1.033	1.662	.272	2.114	.719	1.003	1.722	.392
14	1.710	.630	.886	1.516	.194	1.953	.730	1.018	1.748	.205
15	1.686	.577	.827	1.404	.282	1.821	.643	.962	1.605	.216
16	1.578	.555	.796	1.351	.227	1.834	.650	1.031	1.681	.153
17	1.575	.536	.802	1.338	.237	1.833	.686	.961	1.647	.186
18	1.522	.536	.841	1.377	.145	1.723	.649	.896	1.545	.178
19	1.570	.561	.839	1.400	.170	1.745	.636	.903	1.539	.206
20	1.662	.489	.838	1.327	.335	1.847	.672	.853	1.525	.322
21	1.619	.630	.755	1.385	.234	1.799	.729	.792	1.521	.278
22	1.378	.656	.647	1.303	.075	1.547	.621	.807	1.428	.119
23	1.719	.751	.679	1.430	.289	1.926	.748	.853	1.601	.325
24	2.007	1.115	.708	1.823	.184	2.253	.888	.832	1.720	.533
25	2.183	1.250	.769	2.019	.164	2.451	1.364	.877	2.241	.210
26	2.237	.823	.802	1.625	.612	2.513	1.094	.843	1.937	.576
27	2.218	1.161	.767	1.928	.290	2.491	1.342	.881	2.223	.268
28	2.401	1.416	.713	2.129	.272	2.697	1.581	.912	2.493	.204
29	2.375	1.442	.711	2.153	.222	2.663	1.584	.858	2.442	.221
30	2.253	1.455	.779	2.234	.019	2.526	1.558	.922	2.480	.046
31	2.352	1.405	.734	2.139	.213	2.714	1.630	.887	2.517	.197
32	2.469	1.469	.787	2.256	.213	2.676	1.606	.876	2.482	.194
33	2.681	1.545	.885	2.430	.251	2.727	1.575	.907	2.482	.245
34	2.725	1.682	.839	2.521	.204	2.661	1.660	.812	2.472	.189
35	.....	.....	.....	.....	.....	2.631	1.654	.968	2.622	.009
36	.....	.....	.....	.....	.....	2.642	1.607	.927	2.534	.108
37	.....	.....	.....	.....	.....	2.595	1.581	.942	2.523	.072

[a] Removed at end of thirty-fourth week.

TABLE V.—*Nitrogen metabolism of steers*—Continued

FULL FEED LOT

[Expressed as pounds per period of seven days]

Week No.	Steer 661.[a]					Steer 663.[b]				
	Nitrogen ingested.	Nitrogen excreted.			Balance.	Nitrogen ingested.	Nitrogen excreted.			Balance.
		In urine.	In feces.	In urine and feces.			In urine.	In feces.	In urine and feces.	
1	.....	.....	.....	.....	.....	2.557	0.491	1.469	1.960	0.597
2	.....	.....	.....	.....	.....	2.612	.530	1.449	1.979	.683
3	2.959	0.653	1.641	2.294	0.665	2.837	.573	1.509	2.082	.755
4	2.838	.580	1.632	2.212	.626	2.542	.631	1.571	2.202	.340
5	2.694	.700	1.852	2.552	.142	2.410	.687	1.459	2.146	.264
6	2.797	.667	1.738	2.405	.392	2.542	.705	1.427	2.132	.410
7	2.540	.753	1.664	2.417	.123	2.367	.745	1.319	2.064	.303
8	2.468	.724	1.579	2.303	.165	2.292	.703	1.293	1.996	.296
9	2.591	.724	1.474	2.198	.393	2.418	.746	1.233	1.979	.439
10	2.595	.838	1.327	2.165	.430	2.427	1.154	1.228	2.382	.045
11	2.590	.865	1.509	2.374	.216	1.947	.779	1.122	1.901	.046
12	2.637	.790	1.466	2.256	.381	1.964	.620	1.154	1.774	.190
13	2.549	.837	1.536	2.373	.176	2.115	.606	1.054	1.660	.455
14	2.531	.757	1.498	2.255	.276	2.140	.635	1.232	1.867	.273
15	2.133	.775	1.243	2.018	.115	1.954	.866	.882	1.748	.206
16	1.828	.641	1.024	1.665	.163	1.313	.665	.690	1.355	—.042
17	1.832	.589	.934	1.523	.309	1.621	.445	.842	1.287	.334
18	1.840	.587	1.043	1.630	.210	1.541	.516	.808	1.324	.217
19	2.031	.589	1.068	1.657	.374	1.650	.512	.803	1.315	.335
20	1.778	.748	.915	1.663	.115	1.785	.634	.798	1.432	.353
21	2.064	.659	.987	1.646	.418	1.787	.670	.802	1.472	.315
22	1.741	.719	.944	1.663	.078	1.686	.645	.790	1.435	.251
23	2.434	.725	1.029	1.754	.680	2.118	.886	.848	1.734	.384
24	2.847	1.357	1.051	2.408	.439	2.481	1.309	.905	2.214	.267
25	3.089	1.619	1.103	2.722	.367	2.396	1.499	.746	2.245	.151
26	3.167	1.189	1.091	2.280	.887	2.320	1.408	.695	2.103	.217
27	3.140	1.674	1.126	2.800	.340	2.683	1.672	.845	2.517	.166
28	3.398	1.754	1.159	2.913	.485	2.950	1.683	.772	2.455	.495
29	3.383	1.990	1.104	3.094	.289	2.573	1.815	.670	2.485	.088
30	3.209	1.951	1.133	3.084	.125	1.692	1.625	.551	2.176	—.484
31	3.474	1.886	1.122	3.008	.466	.....	.....	.....	.....	.....
32	3.435	1.873	1.160	3.033	.402	.....	.....	.....	.....	.....
33	3.491	2.069	1.049	3.118	.373	.....	.....	.....	.....	.....
34	3.406	2.056	1.027	3.083	.323	.....	.....	.....	.....	.....
35	3.368	2.023	1.068	3.091	.277	.....	.....	.....	.....	.....
36	3.381	1.920	1.179	3.099	.282	.....	.....	.....	.....	.....
37	3.158	1.930	1.037	2.967	.191	.....	.....	.....	.....	.....

[a] No data obtained for first and second weeks.
[b] Removed at end of thirtieth week.

From the curve showing the live weights of the two steers it is seen that they made considerable gain during the experiment. However, from the eighth to the twenty-second week they made practically no gain. During this time the steers consumed from 0.40 to 0.45 pounds of digestible crude protein daily per 1,000 pounds live weight. In this connection it should be noted that 0.60 pounds of digestible crude protein

is generally accepted as the minimum for maintenance of cattle. The curves for the nitrogen balance show that the amount of protein consumed during this time was not only sufficient for maintenance but that there was a considerable storage of nitrogen. During this time—the eighth to the twenty-second week—the net energy consumption was a little higher than the usually accepted standard. The steers consumed from 6.7 to 7 therms, while 6 therms are considered the requirement for maintenance. In this connection it may be noted that from the thirty-fourth to the thirty-seventh week steer 656, when receiving 6 therms of energy, made a daily gain of ¾ pound per day.

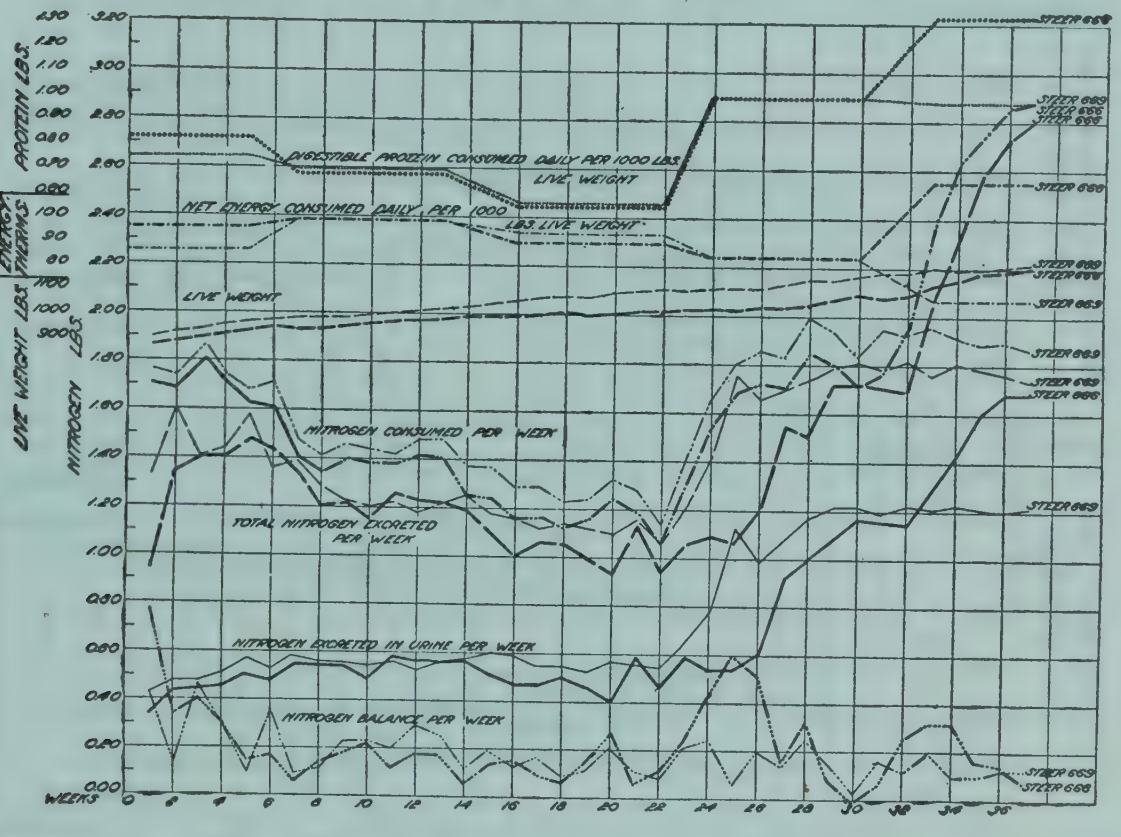

FIG. 2.—Nitrogen metabolism of steers in the one-third feed lot.

Note how nearly parallel are the curves showing the nitrogen consumption, the total nitrogen excretion, and the urinary nitrogen. The curve showing the nitrogen balance, while following the general trend of the nitrogen consumption, is much more irregular. Usually a slight decrease in the amount of nitrogen consumed caused a greater decrease in the amount of nitrogen stored, while a considerable increase in the amount of nitrogen consumed resulted in a much smaller increase in the amount of nitrogen stored.

ONE-THIRD FEED LOT.—Figure 2 shows the same data for the two steers of the one-third feed lot. It will be noted that in the same periods the consumption of digestible protein and net energy was considerably greater for the one-third feed steers than for the maintenance steers. Consequently the increase in live weight was more rapid and more uni-

form. Apparently, however, there is but little relation between the curves showing the live weights and the curves showing the protein and energy consumption.

As with the maintenance lot, the curves showing the nitrogen consumption, the nitrogen excretion, and the urinary nitrogen are more or less parallel from week to week. Though in general the curves showing the nitrogen balance tend to follow the nitrogen consumption, yet there are many instances where they do not. As might be expected, the storage of nitrogen was greater in the one-third feed lot than in the maintenance lot. It also may be noted that in the one-third feed lot the storage of nitrogen was more irregular than in the maintenance lot.

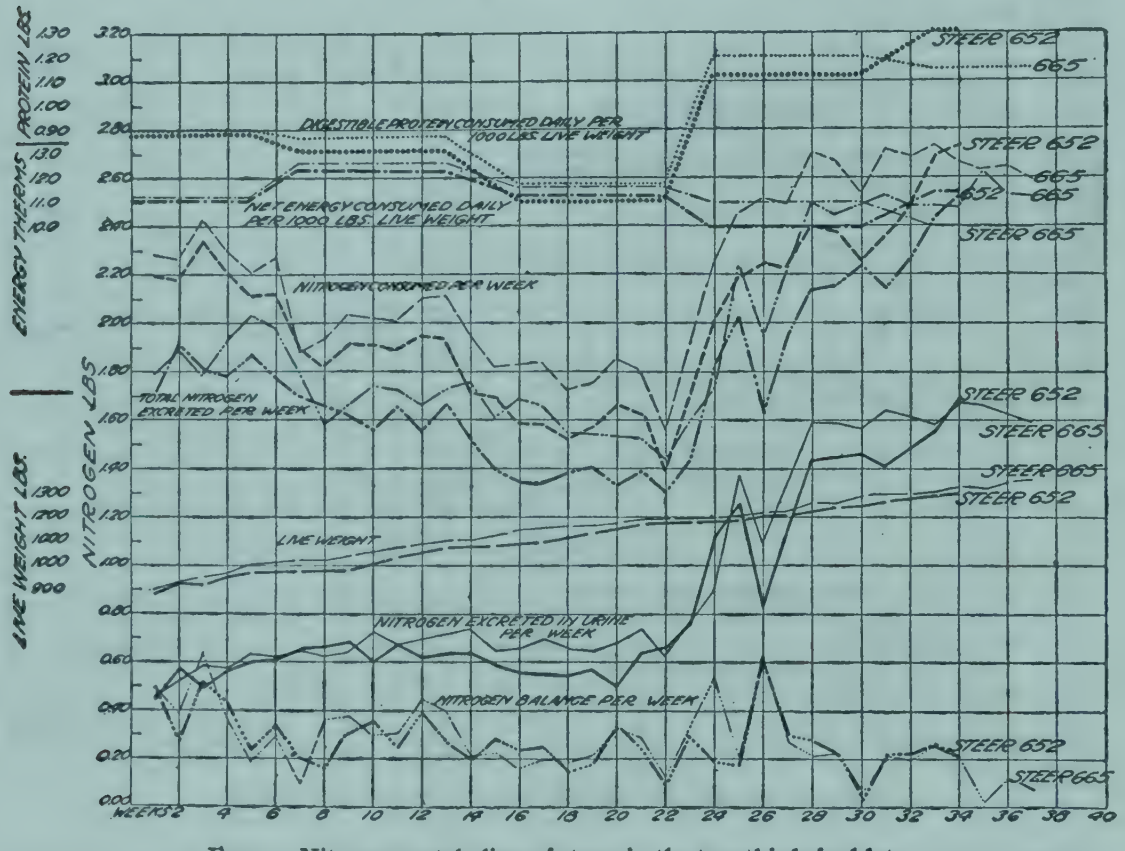

FIG. 3.—Nitrogen metabolism of steers in the two-thirds feed lot.

TWO-THIRDS FEED LOT.—Figure 3 gives the data for the steers of the two-thirds feed lot. It is seen that the consumption of protein and energy was still further increased, resulting in greater gains as shown by the more rapid incline of the live-weight curve.

The curves showing the total nitrogen excretion and the urinary nitrogen again follow the curve of the nitrogen consumption quite closely. The storage of nitrogen again follows the nitrogen consumption more or less closely with numerous irregularities. In general the curve is higher than in either of the lots previously studied.

FULL FEED LOT.—Figure 4 gives the corresponding curves for the full feed lot. It is noted that the consumption of protein and energy was somewhat greater than in the preceding lot, resulting in still better gains

in live weight. Steer 663 of this lot went off feed and had to be removed from the experiment at the end of the thirtieth week.

The relation between the nitrogen consumption and nitrogen balance was about the same as in the preceding lots, with more numerous and more striking irregularities. The nitrogen balance was generally greater than in the two-thirds feed lot, although there were many weeks when the reverse was true.

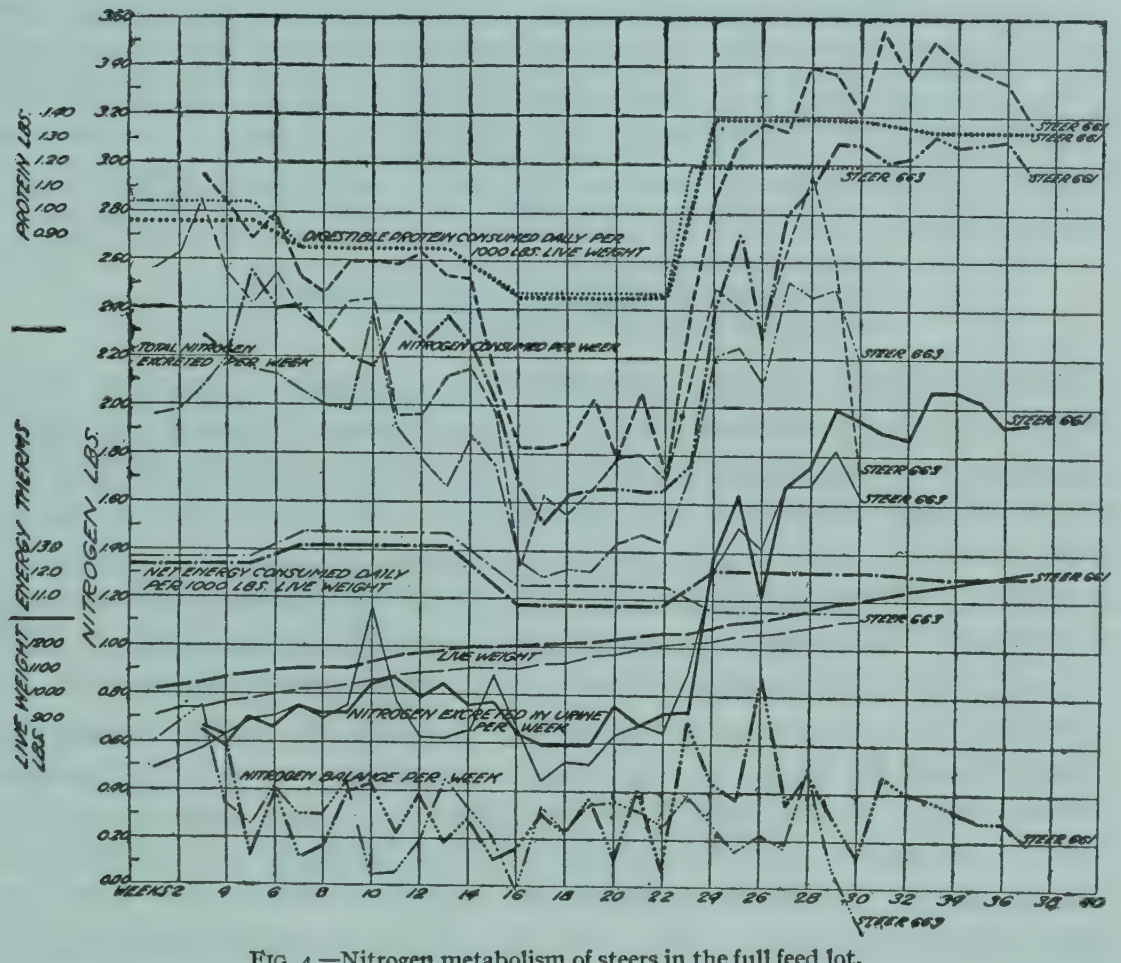
PROTEIN LBS.
ENERGY THERMS
LIVE WEIGHT LBS.
NITROGEN LBS.
DIGESTIBLE PROTEIN CONSUMED DAILY PER 1000 LBS. LIVE WEIGHT
TOTAL NITROGEN EXCRETED PER WEEK
NITROGEN CONSUMED PER WEEK
NET ENERGY CONSUMED DAILY PER 1000 LBS. LIVE WEIGHT
LIVE WEIGHT
NITROGEN EXCRETED IN URINE PER WEEK
NITROGEN BALANCE PER WEEK
STEER 661
STEER 663
WEEKS 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40

FIG. 4.—Nitrogen metabolism of steers in the full feed lot.

PERCENTAGE OF PROTEIN IN GAIN

In an experiment covering the length of time of this one it is reasonably accurate to calculate the percentage of protein in the total gain in live weight from the amount of nitrogen stored and the increase in live weight. This is particularly true when one considers the entire experiment of 37 weeks. The results are given in Table VI.

TABLE VI.—*Percentage of protein in gain*

[Results expressed in percentages]

Period No.	Weeks included in experiment.	Ratio of hay to corn to oil-meal.	Maintenance lot.		One-third feed lot.		Two-thirds feed lot.		Full feed lot.		Average of all steers.
			Steer 650.	Steer 656.	Steer 666.	Steer 669.	Steer 652.	Steer 665.	Steer 663.[a]	Steer 661.	
1.....	1–5	1:1:0	11.02	10.14	13.99	14.42	11.50	10.92	13.87	16.79	12.83
2.....	8–13	1:3:0	(b)	(b)	11.50	15.23	11.30	20.27	10.21	13.41	13.65
3.....	17–22	1:5:0	(b)	(b)	21.20	9.26	11.90	18.75	12.71	24.68	13.08
4.....	25–30	1:4:1	15.17	9.68	18.53	8.33	13.53	12.10	5.18	14.28	12.10
5[c].....	34–37	1:4:1	10.43	22.73	7.31	11.50	[d]11.36	6.09	.....	11.95	11.62
Total..	1–37	.....	14.95	21.15	14.55	13.15	13.52	13.32	11.68	15.37	14.71

[a] Steer 663 removed at end of thirtieth week.
[b] Amount of protein stored greater than gain in live weight.
[c] Steers 650, 666, and 652 were on full feed in period 5.
[d] Steer 652 removed at end of thirty-fourth week.

From this table it is seen that there is no indication that the steers getting the larger amounts of nitrogen and energy showed any larger proportion of protein in their increase in live weight. Neither is there any indication that there was any difference in the percentage of protein in the increase in live weight during any period because of differences in the ration or differences in the age of the steers. Considering the average of all steers for the entire 37 weeks of the experiment, we find that 14.71 per cent of the total increase in live weight was protein (nitrogen × 6.25).

Jordan,[1] in an experiment with steers between the ages of 23 and 33 months, found by means of comparative slaughter tests that the increase in live weight during this time contained 13.57 per cent of protein. Waters, Mumford, and Trowbridge,[2] at the Missouri Experiment Station, found by means of comparative slaughter tests upon steers of similar age and size that the first 500 pounds of gain—that is, the increase in live weight from 748 to 1,248 pounds—contained 11.9 per cent of protein. Thus it is seen that while the method of experimentation used by Jordan and by Waters was entirely different from our own, yet quite similar results were obtained.

PERCENTAGE OF DIGESTED PROTEIN RETAINED

Table VII shows the percentage of the digested protein which was retained in the body by the individual steers. With the exception of the maintenance steers in period 1, there is no indication that the steers receiving larger amounts of digestible protein and net energy stored any more of the protein digested. In period 2 all steers stored less protein than they did in period 1, although there is no distinctive differences between lots. This decrease (from an average of 41.64 per cent

[1] Recalculated by Armsby. (ARMSBY, H. P. NUTRITION OF FARM ANIMALS. p. 372. New York, 1917.)
[2] BULL, Sleeter. PRINCIPLES OF FEEDING FARM ANIMALS. p. 32. New York, 1916.

to 27.41 per cent) may have been due to the smaller amounts of protein received in this period, or to the increase in the age of the steers, or to both. In period 3, when both the protein and energy were decreased, some of the steers stored more and others less protein. However, in period 4, when the protein was practically doubled and the energy slightly decreased, there was a considerable decrease in the percentage of protein stored, except for one steer, No. 666. The results obtained in period 5 are so irregular as to be of little value, although with one exception, steer 650, which was on full feed in this period, the results are usually lower. In general, our results indicate that a smaller percentage of the protein is retained as the age of the animal increases. As already pointed out, however, the protein and energy consumption for different periods varied, and this variation probably materially affects the value of our results.

TABLE VII.—*Percentage of digested protein retained*

Period No.	Weeks included in experiment.	Ratio of hay to corn to oilmeal.	Maintenance lot.		One-third feed lot.		Two-thirds feed lot.		Full feed lot.		Average of all steers.
			Steer 650.	Steer 656.	Steer 666.	Steer 669.	Steer 652.	Steer 665.	Steer 663.[a]	Steer 661.	
1.....	1–5	1:1:0	36.55	36.46	47.73	36.76	42.41	42.85	47.94	42.37	41.63
2.....	8–13	1:3:0	25.25	25.04	23.94	28.80	30.66	34.52	24.20	26.91	27.41
3.....	17–22	1:5:0	25.60	28.56	21.40	19.44	26.07	24.41	34.62	27.83	25.99
4.....	25–30	1:4:1	16.65	9.60	24.23	11.00	17.32	15.23	6.10	19.65	14.97
5[c b].....	34–37	1:4:1	27.87	[c]0.25	9.76	8.72	[d]10.58	5.54	.....	11.90	12.39
Total..	1–37	.....	25.06	18.92	22.93	18.98	23.98	21.70	24.03	23.33	22.37

[a] Steer 663 removed at end of thirtieth week.
[b] Steers 650, 666, and 652 were on full feed in period 5.
[c] Not included in average of all steers.
[d] Steer 652 removed at end of thirty-fourth week.

A study of the results for all steers for the entire 37 weeks of the experiment shows no distinctive differences between lots. In fact individual differences are quite small, considering the nature of the experiment. The results show that the eight steers stored from 18.92 to 25.06 per cent, or an average of 22.37 per cent, of the protein digested.

SUMMARY

(1) The results pertaining to the nitrogen metabolism of eight 2-year-old steers for a period of 37 weeks are given.

(2) Steers maintained a positive nitrogen balance for long periods of time when receiving considerably smaller amounts of digestible protein than are usually considered necessary for maintenance.

(3) Curves showing the nitrogen consumption, the total nitrogen excretion, the urinary nitrogen, and the nitrogen balance are more or less parallel.

(4) Steers receiving larger amounts of nitrogen stored larger amounts.

(5) The amount of nitrogen consumed had no effect upon the percentage of protein in the increase in live weight.

(6) Differences in the ration and in the age of the steers had no effect upon the percentage of protein in the increase of live weight.

(7) An average of the results for eight steers for 37 weeks shows that 14.71 per cent of the increase in live weight was protein.

(8) The amount of protein and energy consumed had no effect upon the percentage of the protein retained.

(9) It is indicated that a smaller percentage of the protein is retained as the age of the animal increases.

(10) An average of the eight steers for 37 weeks shows that 22.37 per cent of the protein digested was stored in the body.

DEVELOPMENT OF THE PISTILLATE SPIKELET AND FERTILIZATION IN ZEA MAYS L.[1]

By EDWIN C. MILLER

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INTRODUCTION

During the past five years considerable time has been given to a cytological study of the pistillate spikelet and flower of the corn plant (*Zea mays*). This work was undertaken with the primary idea of obtaining some facts that could be used in the advanced instruction of students in agriculture, since the cytological work that has been reported for the more common crop plants is limited and fragementary. The lack of investigations of this kind has long been felt not only by those giving instruction to students in botany, agronomy, and plant breeding but also by those who are concerned with investigations in the practical breeding and improvement of crop plants.

REVIEW OF LITERATURE

Crozier (*2*)[2] found that the silk of corn would remain in a receptive condition and grow in length for a long time if pollination was prevented. He also found that it was not alone the forked tip of the silk that was receptive to pollen but that fertilization could be effected by the pollination of the silks after the branched tips had been removed. True (*14*) studied the development of corn, wheat, and oats from the time of fertilization to the maturity of the seed. He described the pistillate flower of corn only in so far as it would be of aid to him in discussing the formation of the caryopsis. Guignard (*4*) described in considerable detail the structure of the ovary and ovule of corn and observed the process of double fertilization but published no drawings of his observations. Poindexter (*11*) described the development of the pistillate spikelet of corn and discussed briefly the early stages in the development of the

[1] Published with the approval of the Director. Contribution from the Department of Botany, Kansas Agricultural Experiment Station, paper No. 31.

[2] Reference is made by number (italic) to "Literature cited," p. 264–265.

embryo and endosperm. Kuwada (*9*) made a cytological study of the pollen mother cells of a number of varieties of corn. He found that there was a considerable variation in the size and number of the chromosomes even in the same race. The haploid number varied from 9 to 12, the higher number as a rule being found in the varieties of sugar corn and the lower numbers in the varieties with more starch. In a later paper (*10*) he reported that the diploid number of chromosomes varied from 20 to 22 in the cells of the root, but that the number was constant for any one plant. Weatherwax (*15–17*) since this work has been in progress has reported extensively on the development, structure, and evolution of both the pistillate and staminate spikelets of the corn plant. Further mention of his work will be made in the discussion of the results reported in the present article.

EXPERIMENTAL METHODS

The varieties of corn used in this work were Pride of Saline, Freed White Dent, and Sherrod White Dent. The material was collected in the field at Garden City, Kans., during the seasons of 1914 to 1917, and at Manhattan, Kans., in 1918. All the material used in this investigation was fixed in medium chrom-acetic solution, washed, dehydrated, cleared in xylol, and embedded in paraffin in the usual manner. The sections for the most part were cut 15 to 20 microns in thickness and stained with safranin, gentian violet, and orange G. The drawings of the developing spikelet were made with the aid of a Bausch and Lomb projection apparatus, and those showing the development of the embryo sac and fertilization were made by the aid of the camera lucida.

In order to study the time elapsing between pollination and fertilization, the young ears were bagged before the silks appeared. After the silks had practically all appeared, they were hand-pollinated with freshly collected pollen. After pollination the ears were again bagged and kept covered until the specimens were collected for fixing. The ears which furnished the material for study were collected at stated hourly intervals after pollination had been made. In this manner the time elapsing between the time of pollination and fertilization could be determined.

For the study of the course of the pollen tube, the silks at certain periods after pollination were cut into short lengths and then tied into small bundles by means of fine threads. These bundles were then fixed and embedded in the same way as the other material. By cutting the bundles lengthwise, a large number of silks for a portion of their length could be obtained in longitudinal section. Since the bundles were taken consecutively from the tip of the silk to the ovary, the course of the pollen tube could be observed in any portion of the silk.

EXPERIMENTAL DATA

MATURE PISTILLATE SPIKELET

The pistillate spikelet of corn at the time the silk emerges from the husk has the appearance in longitudinal section shown in Plate 19. The two empty glumes of the spikelet are thickened at their base but are thin and membranous at their tips. The spikelet bears two flowers, but in most cases one of these aborts, so that in each spikelet there is only one functional flower. Each of the flowers of the spikelet consists of a pistil and three stamens. The stamens in both flowers, however, remain rudimentary, so that the only part of the fertile flower that functions is the pistil. The development and disorganization of the stamens as well as the development and abortion of the pistil of the sterile flower have been described in much detail by Weatherwax (*16*). Each flower is subtended by a lemma or flowering glume. Between the two flowers and adjacent to each other are located the two palets. The palets and lemmas are much shorter and more membranous than the empty glumes. With the exception of the pod corns, the bracts of the spikelet cease growth at the time of fertilization and thus never completely inclose the ovary. The bracts remain at the base of the grain and form the chaff of the cob. If fertilization is not effected, however, the bracts of the spikelet continue to grow in length and will completely inclose the ovary of the fertile flower. In pod corn, the bracts continue to grow after fertilization and completely inclose the mature grain.

When the spikelet is mature the lodicules of the fertile flower are not present or are not easily seen. According to Weatherwax (*16*) the lodicules in early stages of growth are present in both flowers, but those of the functional flower are crowded out before it is mature, while those of the sterile flower remain intact and can readily be observed even when that flower has a functional pistil.

The pistil of the fertile flower consists of the ovary and the elongated style or "silk." The silk is unevenly cleft at its tip, and this branched portion has been termed the stigma of the pistil by most authors. A small rounded knob or protuberance is located at the top of the ovary near the base of the silk. In the center of this knob is a funnel-shaped depression, apparently leading to the cavity of the ovary. However, an examination of a section through this region shows that the depression is only superficial and that the opening which at one time led to the cavity of the ovary has been closed. The cells composing the wall of this cavity have never completely united (Pl. 19, sc). This incomplete union of the wall of the ovary was noticed by True (*14*) but was first termed the stylar canal by Guignard (*4*). The origin of this canal will be discussed in detail when the embryonic development of the spikelet is considered.

The ovule is of a modified campylotropous type and is attached by approximately one-third of its circumference to the bottom of the cavity of the ovary. The outer coat of the ovule is incomplete and extends about half way around it. The outer coat for its whole length, with the exception of a short distance at the base, is free from the inner coat. The inner coat fits closely to the ovule and covers it completely, except in the region of the micropyle. Each coat of the ovule is approximately two cells in thickness, except in the region of the micropyle and the stylar canal where the coats may be from three to four cells in thickness. The outer coat forms a wedge-shaped projection which extends into the inner depression of the stylar canal. The inner coat also often shows such a projection, but it is never so marked as that in the outer coat (Pl. 19, ovc). This projection of the outer coat into the stylar canal has been observed by both Guignard (*4*) and Weatherwax (*16*).

The two fibro-vascular bundles of the silk traverse the walls of the ovary and unite at its base with the bundles that supply the various elements of the spikelet. Extending from each of the fibro-vascular bundles of the silk to the cavity of the ovary is a bundle of elongated cells that are rich in protoplasm and resemble very closely the sheath cells of the fibro-vascular bundles of the silk. Through these sheathlike cells the pollen tube travels to the cavity of the ovary after it leaves the sheath cells of the fibro-vascular bundles of the silk (Pl. 19, vbs, bsc).

DEVELOPMENT OF THE PISTILLATE SPIKELET

The spikelets are borne on the cob in double rows, because the spikelets are paired; and since each spikelet has only one functional flower, an even number of rows of grains is produced. It has been observed by Kempton (*7*), Stewart (*12*), and Weatherwax (*15*) that frequently in certain varieties both flowers of the spikelet may function, and thus two grains may be produced to each spikelet instead of one. In these varieties the grains do not always occur in regular rows on the cob but may be more or less irregularly arranged. This is due to the fact that the development of two grains in a spikelet tends to crowd the other grains in that region more or less out of alignment.

The origin of the paired spikelets is best observed by a study of the cross section of a very young cob. Such a cross section of the tip shows that it is composed of undifferentiated or embryonic cells (Pl. 20, A). A short distance back of the tip numerous projections or protuberances appear on the periphery of the cob. Each of these projections is a rudiment or primordium from which a pair of spikelets will develop (Pl. 20, B). Soon after the formation of these rudiments, each one becomes equally divided (Pl. 20, C), and from each half a spikelet develops (Pl. 20, D). The progressive development of a spikelet from its primordium is best studied in longitudinal section. The appearance of the embryonic cells

of the tip of a young cob in longitudinal section (Pl. 21, A) differs a little from that in cross section (Pl. 20, A).

A longitudinal section of the rudiment of a spikelet just after its appearance shows that it is composed of embryonic cells and has the general appearance of the tip of the young cob. The first differentiation to appear on the rudiment of the spikelet is the lower empty glume (Pl. 21, B). The primordium of the upper empty glume soon appears (Pl. 21, C), so that at a little later stage the two developing glumes have practically the same appearance (Pl. 21, D). The primordia of the two lemmas or flowering glumes are the next to appear (Pl. 22, A), while directly following, or frequently at the same time, the rudiments of the sterile flower and of the stamens of the fertile flower become visible. The palet of the fertile flower at this time also begins to show differentiation (Pl. 22, B), but the palet of the sterile flower does not appear until considerably later.

The primordium of the carpellate leaf or ovary wall of the fertile pistil begins to show in a short time after those of the palet and stamens of the fertile flower appear (Pl. 23, A). At this time the cells that are to compose the fibro-vascular bundles of the lower part of the spikelet begin to differentiate. The carpel grows unevenly so that when the side adjacent to the lemma extends almost one-third around the young ovule the opposite side has scarcely begun to develop (Pl. 23, A, c, c′). This more rapidly growing portion of the carpel increases in width toward the tip so that it becomes from two to three times wider than the base (Pl. 23, B). This widened portion of the carpel is composed of numerous embryonic cells which later rapidly elongate to form the silk (Pl. 24, A). When the silk is elongating, the wall of the ovary has grown up around the ovule and has almost inclosed it with the exception of a small opening toward the top (Pl. 24, A, sc). This opening has been termed the stylar canal. It, however, does not long remain open, for by the time the silk is ready for pollination, the edges of the carpel have come in close contact but have not grown together (Pl. 25, B).

About the time the silk begins to elongate, the ovule begins to invert. The cells of the ovule on the side adjacent to the palet increase in number and elongate more rapidly than those on the opposite side, thus causing the end of the ovule to turn downward (Pl. 24, B). The megaspore mother cell appears about the time the ovule begins to turn, and frequently the embryo sac has reached the 2-celled stage by the time the ovule has become completely inverted. The ovule coats grow rapidly when the ovule begins to curve, so that by the time it has reached its final position they have reached their full development (Pl. 25, A).

DEVELOPMENT OF THE EMBRYO SAC

About the time the ovule begins to invert, the differentiation of the megaspore mother cell becomes apparent (Pl. 24). No disorganization of any of the megaspores was noted in the three varieties of corn studied

in this experiment, although approximately 50 observations were made. This fact was also observed by Weatherwax (*16*) in the varieties of corn studied by him, so it seems to be the rule that all four megaspores function. In wheat (*Triticum vulgare*), however, Koernicke (*8*) and Jensen (*6*) report that only one megaspore functions. The same has been observed by Cannon (*1*) for wild oats (*Avena fatua*).

The megaspore mother cell increases in size until it becomes about twice as broad and from four to five times as long as the vegetative cells of the ovule (Pl. 26, A, B). The developing embryo sac remains approximately the same size as the megaspore mother cell until the eight cells are formed. At that time it has elongated but slightly while its breadth has increased to two or three times that of the megaspore mother cell (Pl. 27, B). The two polar nuclei migrate and come in contact with each other a short distance above the egg but do not fuse before fertilization takes place (Pl. 27, B, C). In scores of cases where pollination had been prevented the two polar nuclei were observed standing apart a week or more after the embryo sacs were ready for fertilization.

MATURE EMBRYO SAC

When the embryo sac is mature, it is approximately four times as long and about twice as wide as when it first reaches the 8-celled stage. It reaches its maximum size about the time the silk emerges from the husk. The antipodals begin to divide almost immediately after the 8 cells are formed, so that one very rarely finds an embryo sac that shows only 8 cells. The antipodals increase in number, apparently by indirect cell division, until they number from 24 to 36 cells at the time of anthesis. These cells often have indistinct walls, and frequently there are two nuclei to each cell. These cells are closely crowded together and give the appearance of a rather definite tissue (Pl. 28, ant). This behavior of the antipodals is characteristic of the grasses and has been noted by numerous investigators since the time of Hofmeister (*5*). Golinski (*3*) in his work with the stamens and pistil of wheat studied the antipodals with especial care in order to determine whether they played any part in the formation of the endosperm and established the fact that these cells remain intact until they are crowded out by the growing endosperm (Pl. 32, B).

The egg increases in size until its width is almost half that of the embryo sac. It is decidedly balloon-shaped and becomes aveolar in appearance. The synergids are more or less lunar-shaped and are considerably longer than the egg. They have dense cell contents and take the stain much deeper than the egg. (Pl. 28, e, sy). The nuclei of the synergids may disintegrate before fertilization or may remain clear and distinct until it has taken place. In most cases the synergids do not remain long intact after the egg is ready for fertilization. Where

fertilization is delayed they lose their identity and can not be distinguished from the surrounding cytoplasm.

The polar nuclei are embedded in a strand of cytoplasm that extends from the antipodals to the egg, while the greater part of that portion of the embryo sac is taken up by two large vacuoles. The nucleoli of the polar nuclei are the largest in the embryo sac. The two polar nuclei remain in close contact but do not fuse until fertilization has taken place.

SILK AND THE POLLEN TUBE

The end of the silk is cleft into two branches of unequal length. This branched portion of the silk has been termed the stigma by most authors in their description of the corn flower (Pl. 29, A). The silk, however, is receptive to pollen for at least the greater portion of its length; so it would appear that Weatherwax (*16*) is correct in asserting that the term stigma can be applied to the branched tip of the silk only in a morphological sense and not with the understanding that it is the only portion of the pistil on which the pollen grains may germinate.

Numerous hairs are borne on the silk in rather definite areas for its entire length (Pl. 29, A). These hairs appear for the most part on the edges of the silk and are more numerous near its tip than farther down. The hairs may be branched or unbranched and the upper ends of the cells that compose them stand out from the hair (Pl. 29, B), thus forming a rough surface upon which the pollen grains easily lodge. The origin and development of these hairs have been described in detail by Weatherwax (*15*), who observed that each hair originates from a single epidermal cell of the silk.

Two fibro-vascular bundles extend the entire length of the silk and terminate in the branched tip (Pl. 29, A). A cross section of the silk shows that it is grooved on both its upper and lower surfaces and that the vascular bundles are located near its edge (Pl. 30, A). Each bundle contains from three to six xylem elements (Pl. 30, B). The conducting tissue of the fibro-vascular bundles is surrounded by narrow, elongated cells that are characterized by very dense cytoplasmic contents and elongated flattened nuclei (Pl. 30, C). It is between these dense cells that the pollen tube travels down the silk.

The pollen grains vary in shape from spherical to ellipsoidal, and each grain has a germ pore (Pl. 29, C). The protoplasm of the pollen grain is very dense, and often it is difficult to distinguish the nuclei. The two sperm nuclei are formed before the pollen is shed (Pl. 32, A). This supports the statement of Strasburger (*13*) that the division of the generative nucleus in the pollen grain is a constant character for all the grasses.

A few hours after the pollen grains lodge on the hairs of the silk, the pollen tube emerges from the germ pore (Pl. 29, D). Three ways have

been observed by which the pollen tube may gain access to the sheath cells of the fibro-vascular bundles of the silk. Shortly after the pollen tube appears, it may penetrate a hair and through it gain entrance to the fibro-vascular bundle region (Pl. 29, D); or the tube may continue down the outside of hair to its base and then enter the silk and penetrate to the cells surrounding the bundle. Frequently pollen grains that fall directly on the smooth portion of the silk germinate, and the pollen tube penetrates the silk. These instances, however, are exceptions. Practically all pollen tubes that function are from pollen grains that fall on the hairs of the silk.

The end of the pollen tube is greatly enlarged as it pushes its way between the dense sheath cells of the bundle (Pl. 29, E). In its passage down the silk the tube causes but very little disturbance in the position of the cells, so that after the tube disappears the cells quickly return to their normal form and position. The pollen tube, so far as I have observed, does not extend the full length of the silk at any time. It is very difficult to locate it a short distance back of its growing region. It appears that the older portions of the tube are absorbed by the surrounding cells, while the growing part of the tube apparently is nourished by the dense sheath cells. Arriving at the base of the silk, the pollen tube pushes its way between the sheathlike cells that extend from the bundle of the silk to the cavity of the ovary (Pl. 19, vsc). After it enters the ovary cavity the tube twists and coils in its passage along the coats of the ovule until it reaches the micropyle. After passing through the micropyle, the tube works its way between the cells of the ovule and enters the embryo sac (Pl. 28, pt). The protoplasm of the pollen tube is very dense, so that it is very difficult to locate the sperm nuclei. I have never observed them in the tube except after it had entered the embryo sac.

If pollen is supplied abundantly, a great number of pollen tubes start to grow down the bundle regions of each silk. However, as one examines the silk from the tip downward, the number of pollen tubes becomes smaller and smaller, so that when the cavity of the ovary is reached only one pollen tube is to be observed. In nearly a hundred observations no more than one pollen tube was seen in each ovary cavity.

The growth of the pollen tubes is very rapid, and under ordinary conditions they reach the embryo sacs of all the ovules on the ear in 24 hours after pollination. In order to do this the longest tubes must grow in that time approximately 6 inches, a distance that equals 1,500 times the diameter of the pollen grain.

FERTILIZATION

After the entrance of the pollen tube into the embryo sac, it expands so that the width of its tip is approximately one-third that of the embryo sac. The pollen tube extends into the embryo sac until the tip is near the polar nuclei. The wall of the tube is dissolved, giving the nuclei free access to the embryo sac. One of the sperm nuclei fuses with the egg and another with one of the polar nuclei (Pl. 31). The two polar nuclei fuse at the time the sperm nucleus enters one of them or shortly afterwards. Traces of the pollen tube in the embryo sac remain for a long time and do not disappear until crowded out by the developing endosperm and embryo. Fertilization takes place in from 26 to 28 hours after pollination, or in a few hours after the pollen tube reaches the embryo sac.

DEVELOPMENT OF THE EMBRYO AND ENDOSPERM

Almost immediately after fertilization, the endosperm nucleus begins to divide; and in 10 to 12 hours the nuclei of the endosperm may number 20 or 30, arranged around the periphery of the embryo sac (Pl. 32, A). Many of the nuclei have two nucleoli. The nucleus of the fertilized egg does not divide very rapidly. When the nuclei of the endosperm number as high as 20 or 30, the egg nucleus has just undergone its first division (Pl. 32, A). The cells of the endosperm increase very rapidly, and within 36 hours after fertilization they completely fill the embryo sac (Pl. 32, B). The antipodals remain intact and increase in number but are soon crowded out by the encroaching endosperm cells. By the time the endosperm completely fills the embryo sac the embryo consists of only from 14 to 16 cells (Pl. 32, C).

SUMMARY

In a study of the pistillate spikelet and the process of fertilization in the corn plant (*Zea mays*) the following facts were noted:

Embryo sac.—In the formation of the embryo sac there is no disorganization of the megaspores, and all four function. The three antipodal cells rapidly increase in number, apparently by indirect cell division, until they number from 24 to 36 at the time the embryo sac is mature. These cells have rather indistinct cell walls and frequently contain two nuclei. The two polar nuclei come into position just above the egg and remain in close contact with each other but never fuse before fertilization has taken place. The egg becomes reticulate, stains very lightly, and is decidedly balloon-shaped.

Pollen tube.—Practically all the pollen tubes that function come from the pollen grains that lodge on the hairs of the silk. The tubes may enter the hairs directly and through them gain access to the interior of the silk, or they may follow the hairs to their base and then penetrate the silk. After the pollen tubes are once inside the silk they work their

way between the cells to the fibro-vascular bundles. Each silk has two fibro-vascular bundles. These bundles are surrounded by sheath cells which are characterized by their extremely dense contents and large, flattened nuclei. It is between these cells that the pollen tube travels down the silk. Arriving at the base of the silk, the pollen tube works its way between the sheathlike cells that extend from the fibro-vascular bundle of the silk to the cavity of the ovary. The tube enters the ovary cavity and twists and coils in its passage along the ovule coat until it reaches the micropyle. The tube then pushes between the cells of the ovule until it reaches the embryo sac. The growth of the pollen tubes is very rapid, so that they reach the embryo sacs of all the ovules of the ear in 24 hours after pollination. To do this some of the tubes must grow a distance of approximately 6 inches in the course of the 24 hours. The pollen tubes apparently do not extend the full length of the silk at any given time but are absorbed a short distance back of their tip by the cells between which they pass. A great number of tubes start down a given silk; but the number of tubes becomes less and less as the base of the silk is approached, so that by the time the cavity of the ovary is reached only one tube is to be observed. The two sperm nuclei are formed in the pollen grain before the pollen tube appears.

FERTILIZATION.—The pollen tube enters the embryo sac and pushes its way upward until its tip is near the polar nuclei. The tip of the tube expands until it is approximately one-third the width of the embryo sac. The wall of the tube seems to dissolve, giving the sperm nuclei access to the embryo sac. One of the sperm nuclei fuses with the egg, and at about the same time the other fuses with one of the polar nuclei. The two polar nuclei fuse at the time the sperm nucleus enters one of them or shortly afterwards. The pollen tube persists in the embryo sac until it is crowded out by the developing endosperm and embryo. Fertilization occurs in from 26 to 28 hours after the silks have been pollinated.

ENDOSPERM AND EMBRYO.—The endosperm nucleus soon divides, and in from 10 to 12 hours after fertilization the endosperm nuclei may number as high as 30, arranged around the periphery of the embryo sac. Within 36 hours after fertilization the cells of the endosperm completely fill the embryo sac. The nucleus of the ferilized egg does not divide for some time, so the endosperm may number 20 or more cells before the first division of the egg takes place. When the cells of the endosperm completely fill the embryo sac, the embryo numbers only 14 to 16 cells.

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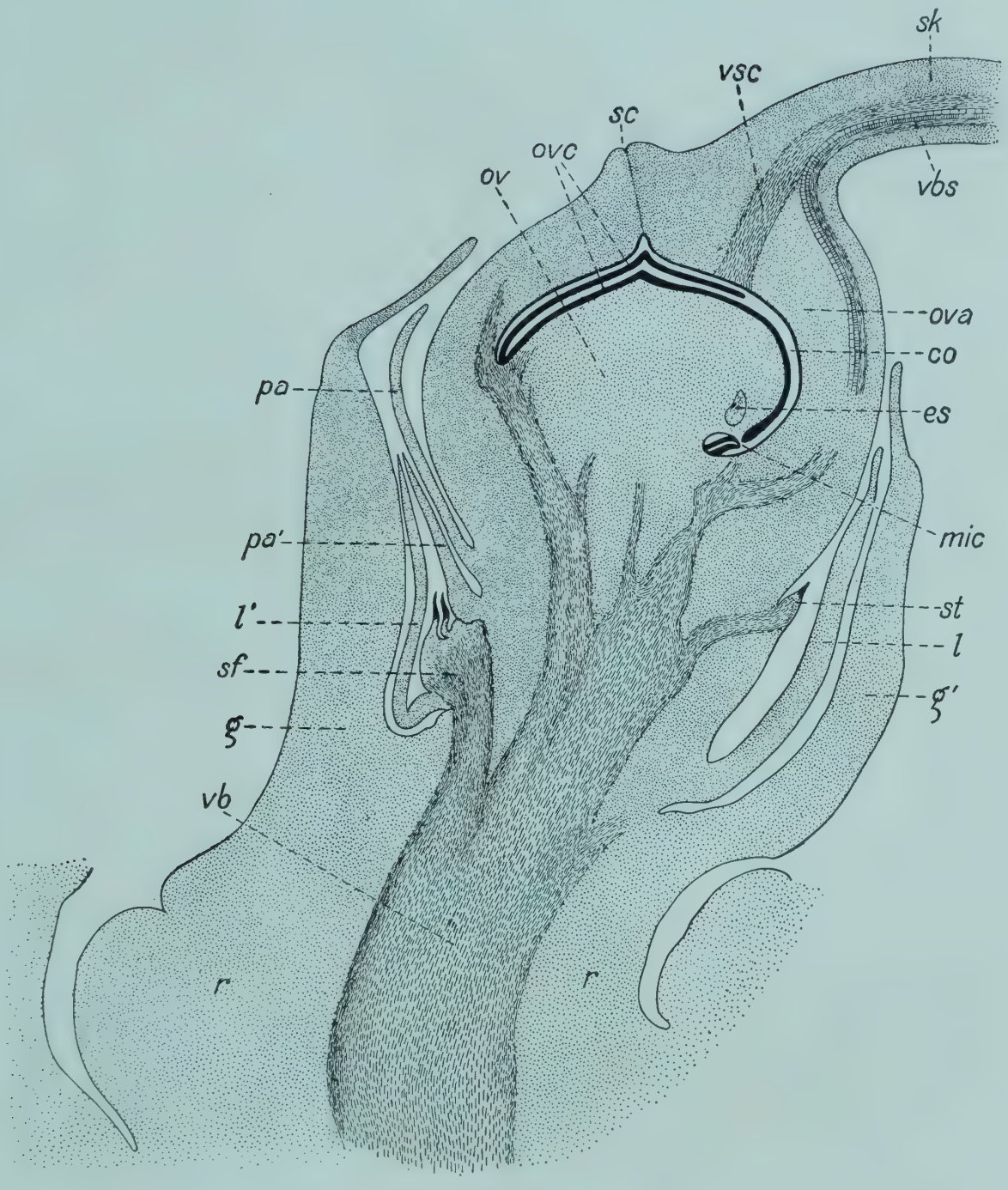
PLATE 19

Longitudinal section of the pistillate spikelet of corn at the time the silk is ready for pollination: r, rachilla; g, lower empty glume; g′, upper empty glume; l, lemma or flowering glume of the fertile flower; l′, lemma or flowering glume of the sterile flower; pa, palet of the fertile flower; pa′, palet of the sterile flower; sf, sterile flower; st, rudimentary stamen of the fertile flower; ova, ovary of the pistil; co, cavity of the ovary; sk, silk or style; sc, stylar canal; vbs, one of the fibro-vascular bundles of the silk.

Through the sheath cells that surround the bundle the pollen tube travels down the silk; vsc, sheathlike cells through which the pollen tube travels from the vascular bundle to the cavity of the ovary; vb, fibro-vascular bundles that supply the parts of the spikelet; ov, ovule; ovc, ovule coats; mic, micropyle; es, embryo sac. × 45.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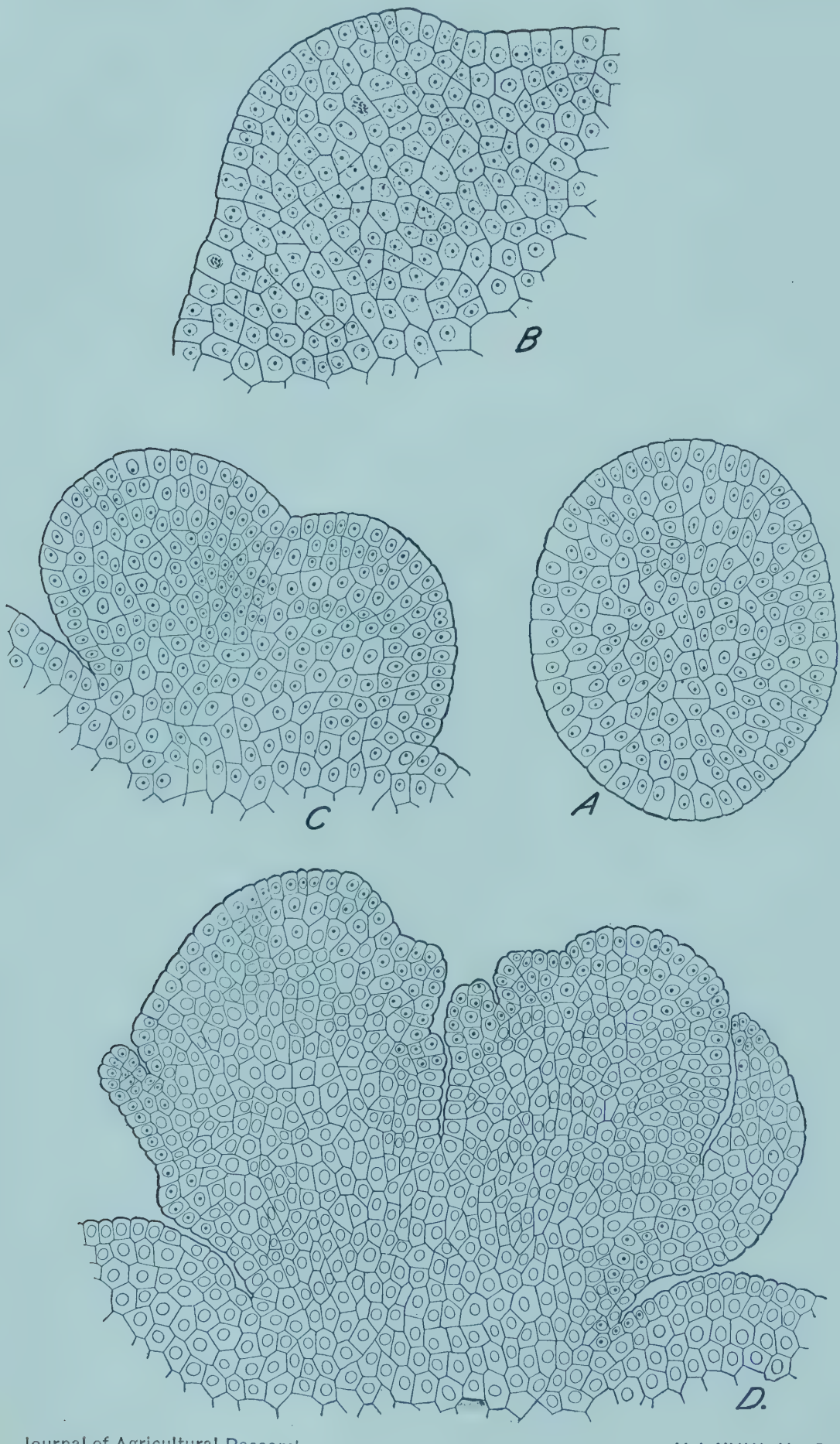

B
C
A
D.

PLATE 20

A.—Cross section of the tip of a very young cob. × 300.

B.—Portion of a cross section of a young cob just back of the tip, showing the rudiment or primordium from which a pair of spikelets will develop. × 300.

C.—Cross section of a rudiment at the beginning of its division into equal parts. × 300.

D.—Cross section of a pair of spikelets in the process of development. × 300.

PLATE 21

A.—Longitudinal section of the tip of a young cob. × 300.

B.—Longitudinal section of the rudiment or primordium of a spikelet just back of the tip of a young cob: g, primordium of the lower empty glume. × 300.

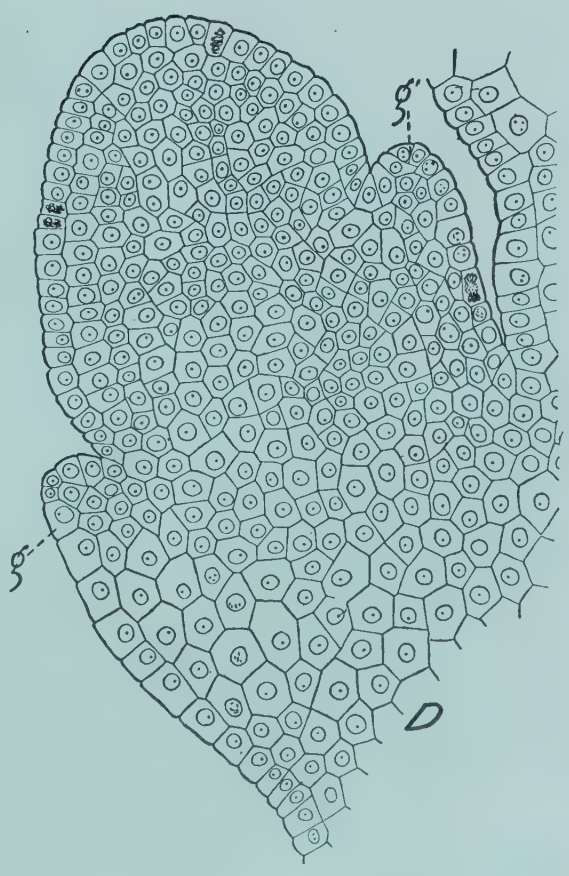
C.—Longitudinal section of the developing spikelet, showing the primordia of the lower and upper empty glumes: g, lower empty glume; g′, upper empty glume. × 300.

D.—Longitudinal section of the developing spikelet at a little later stage than C: g, lower empty glume; g′, upper empty glume. × 300.

A

g
B

g'
g
C


g''
g
D

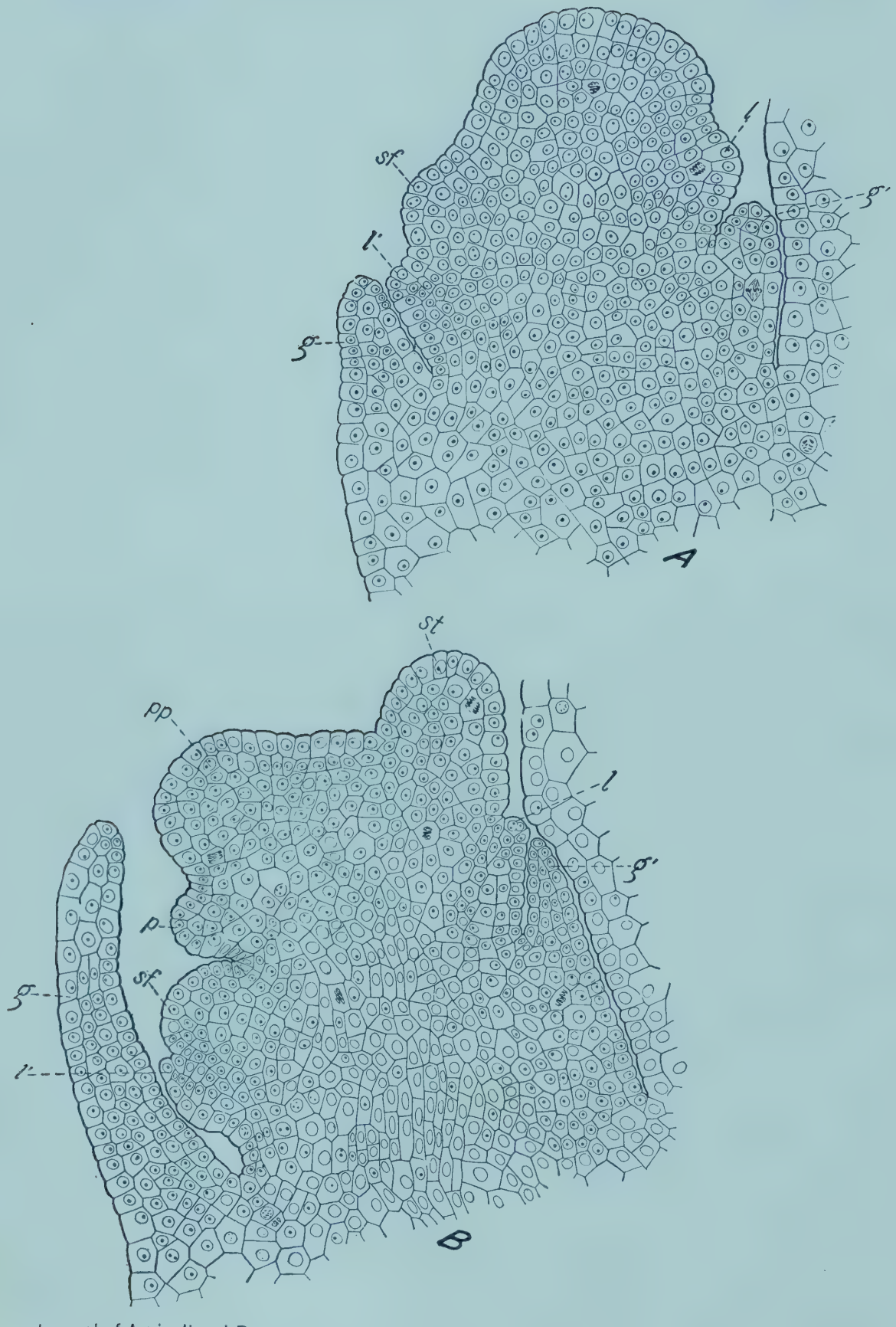

sf
l
s'
s
A
St
pp
l
s'
p
sf
s
l
B

PLATE 22

A.—Longitudinal section of the developing spikelet: g, lower empty glume; g′, upper empty glume; l, primordium of the lemma or flowering glume of the fertile flower; l′, primordium of the lemma or flowering glume of the sterile flower; sf, primordium of the sterile flower. × 300.

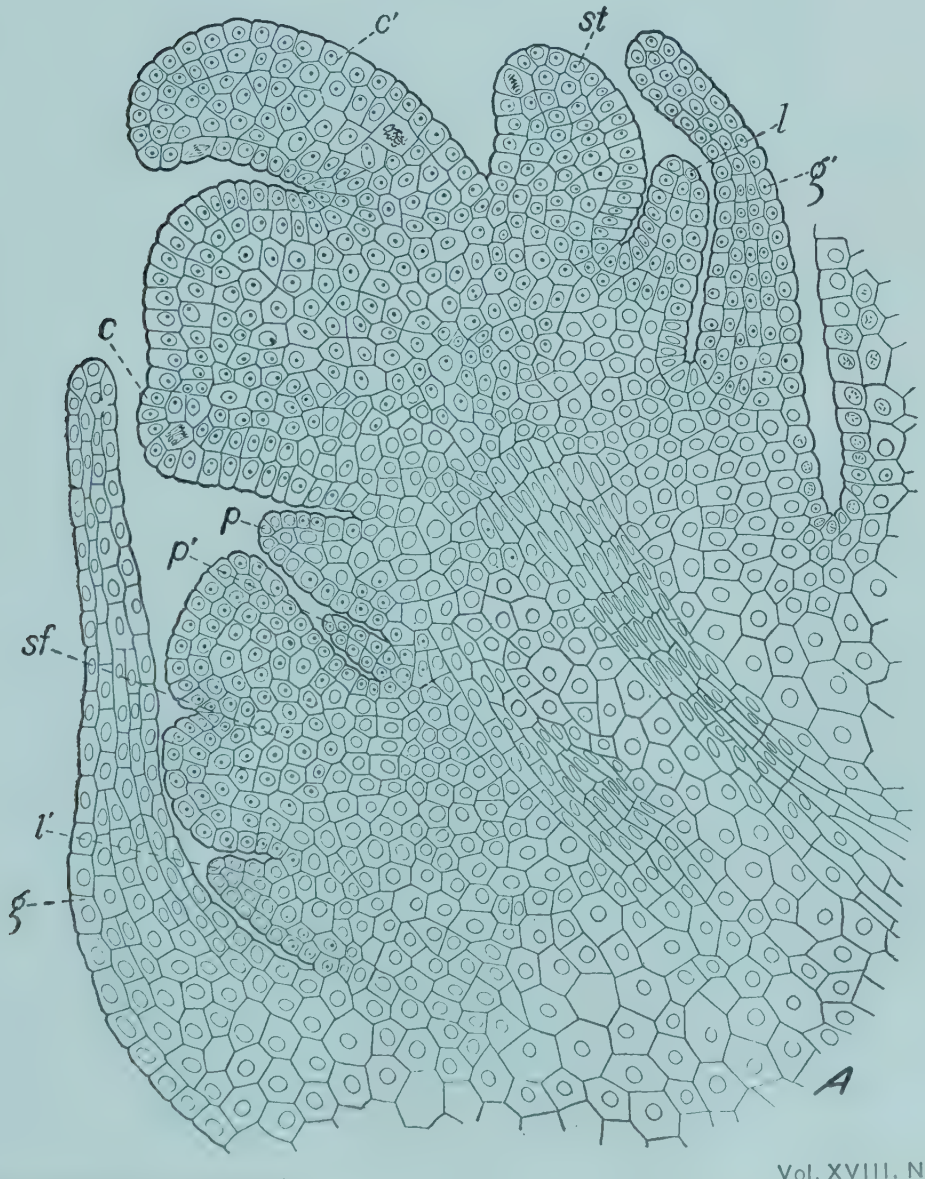
B.—Longitudinal section of the developing spikelet: g and g′, empty glumes; l and l′, lemmas or flowering glumes; sf, primordium of the sterile flower; st, stamen of the fertile flower; p, palet of the fertile flower; pp, primordium of the pistil. × 300.

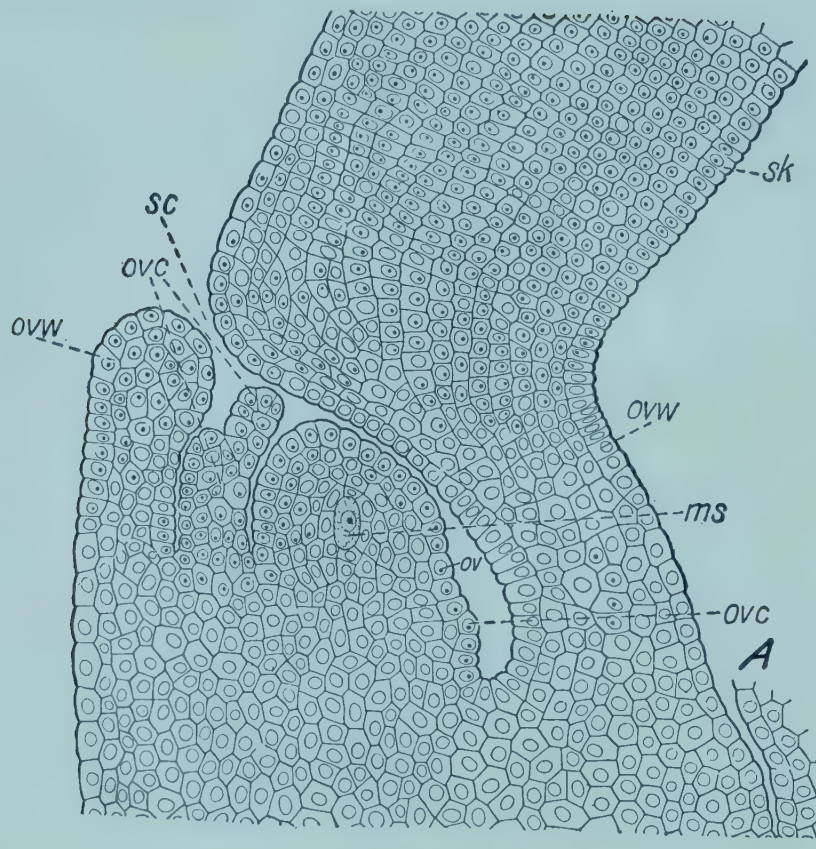
PLATE 23

A.—Longitudinal section of the developing spikelet at the time the carpel or ovary wall has begun to develop: g and g′, empty glumes; l and l′, lemmas; sf, sterile flower; p and p′, palets; st, stamen; c and c′, rudiment of the carpel or ovary wall; c′ is the more rapidly growing part of the carpel. × 300.

B.—Longitudinal section of a developing ovary: c and c′, developing ovary walls; c′ is the portion of the carpel from which the style or silk will develop; ov, ovule; ovc, primordium of the inner ovule coat. × 300.

c
ov
ovc
c
B


c'
st
l
g'
c
p
p'
sf
l'
g
A


sk
sc
ovc
ovw
ovw
ms
ov
ovc
A

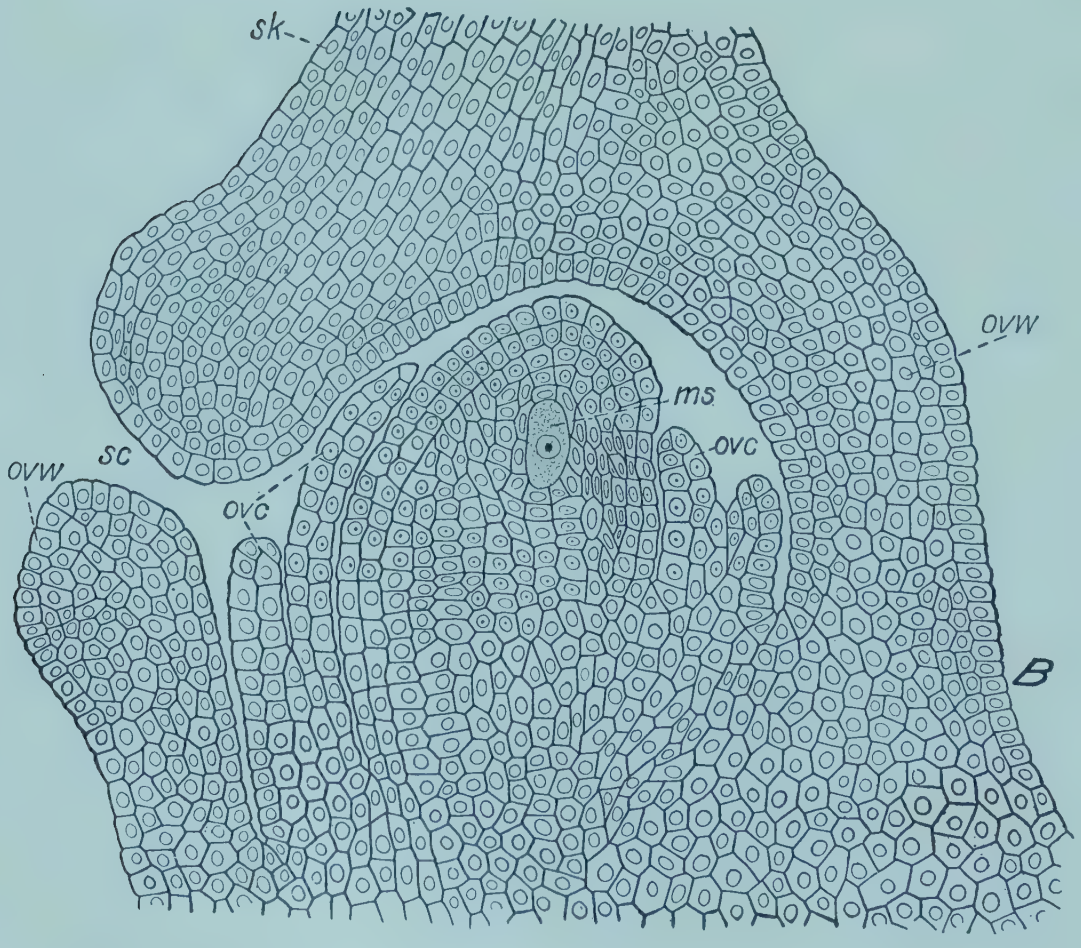

sk
ovw
ms
ovc
ovw
sc
ovc
B

PLATE 24

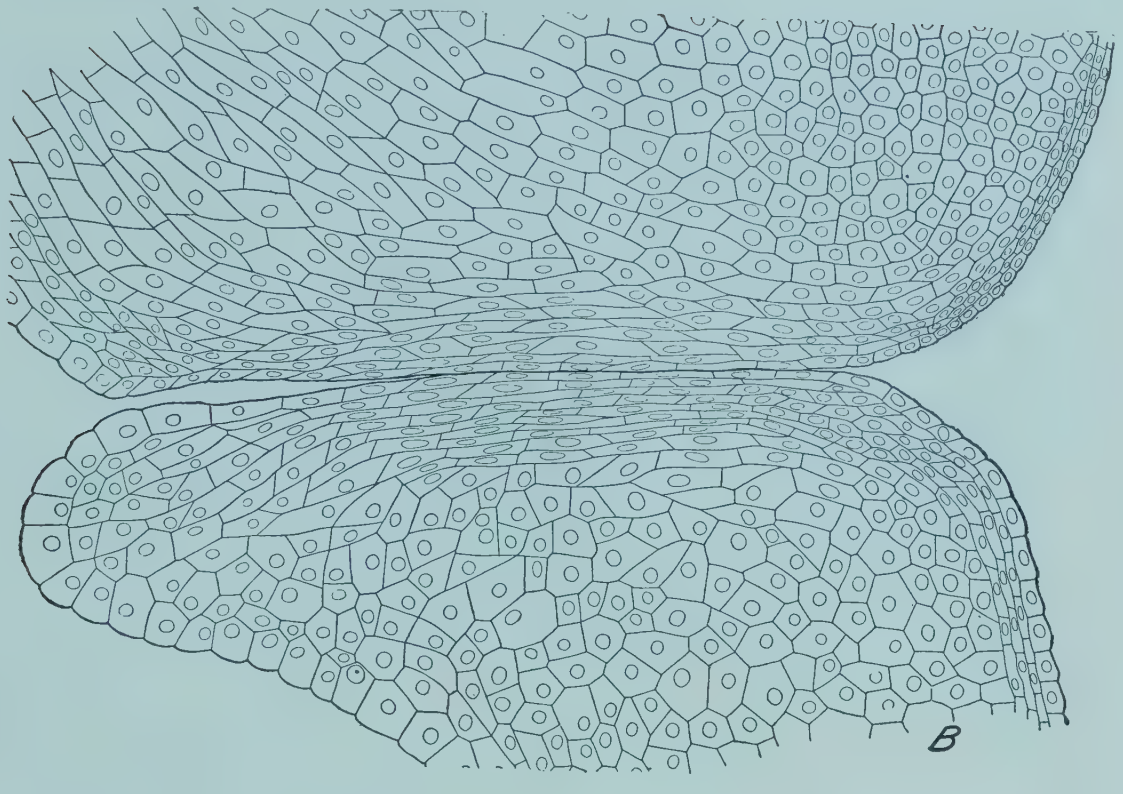
A.—Longitudinal section of the fertile pistil at the time the silk has started to elongate: ovw, ovary wall; sc, stylar canal; ovc, ovule coats; ov, ovule; ms, megaspore mother cell; sk, silk. × 300.

B.—Longitudinal section of the fertile pistil at the time the ovule has started to invert: ovw, ovary wall; sc, stylar canal; sk, silk; ovc, ovule coats; ms, megaspore-mother cell. × 300.

PLATE 25

A.—Longitudinal section of the inverted ovule: ov, ovule; ovc, ovule coats; es, embryo sac; mic, micropyle; co, cavity of ovary. × 250.

B.—Section through the stylar canal, showing its structure shortly before the silk emerges from the husk. The union of the two edges of the carpel will eventually be more complete near the top than is here shown. × 250.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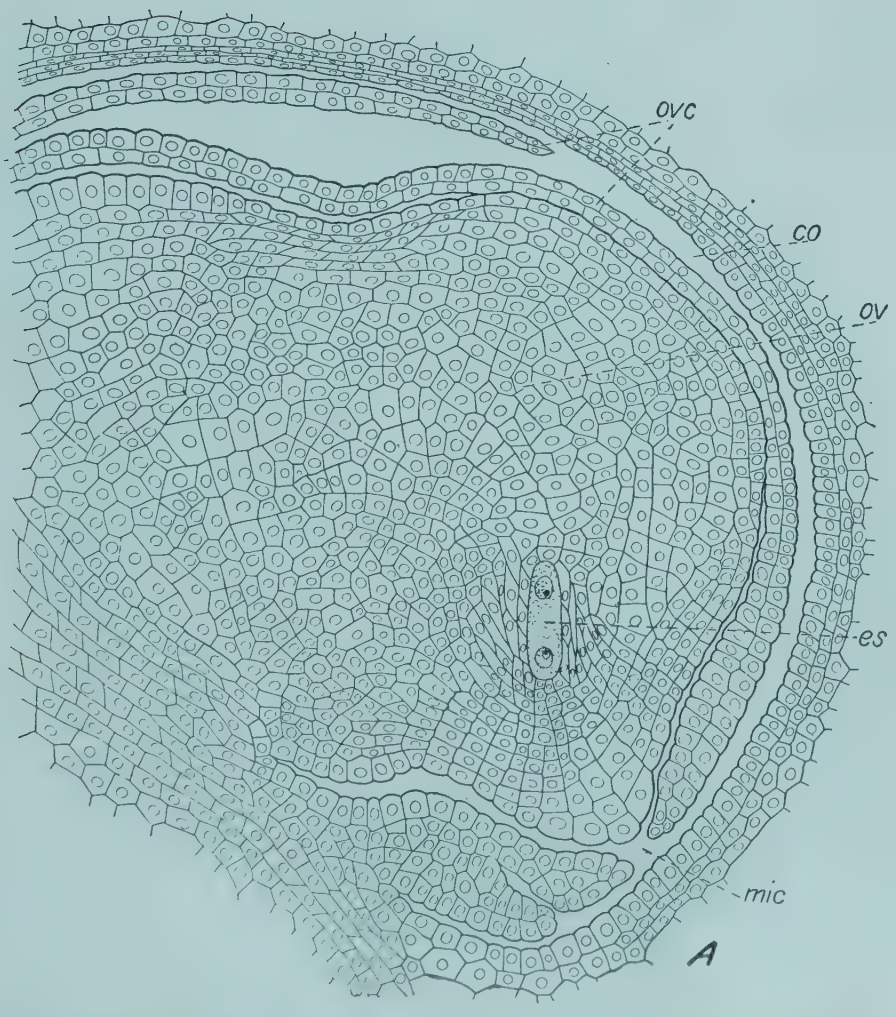
ovc
co
ov
es
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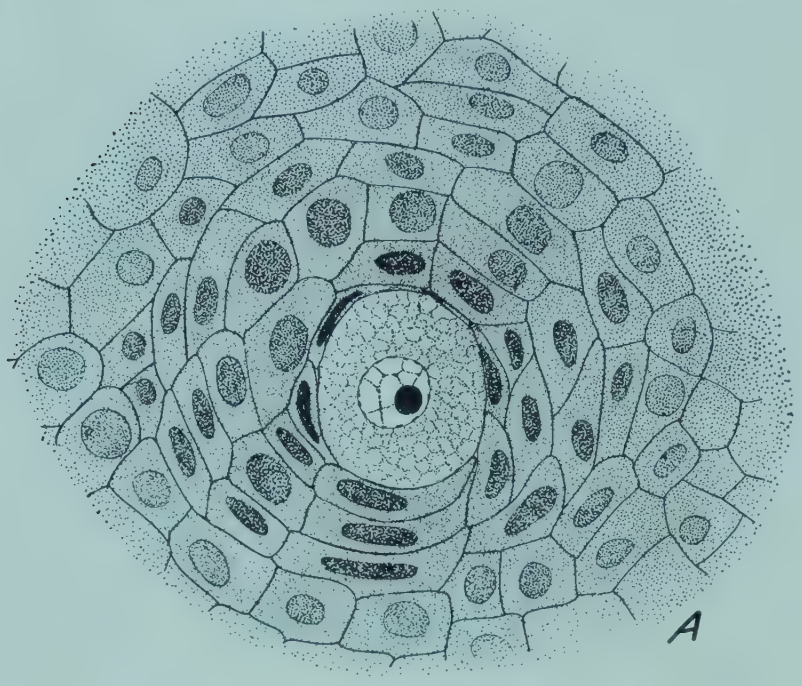
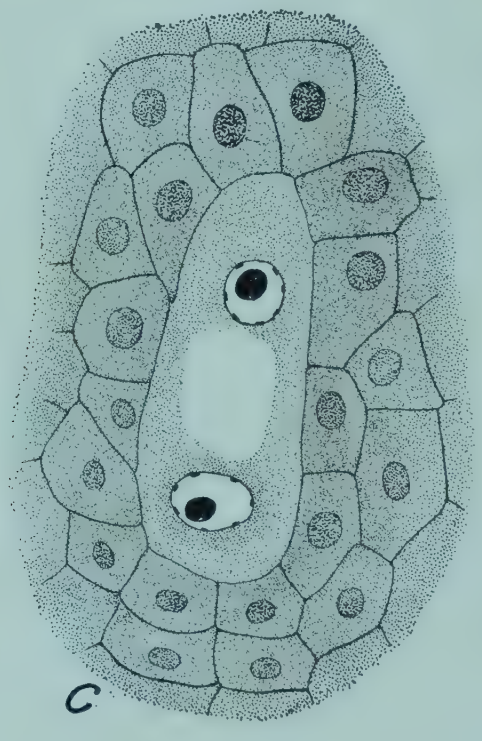
A


A


C

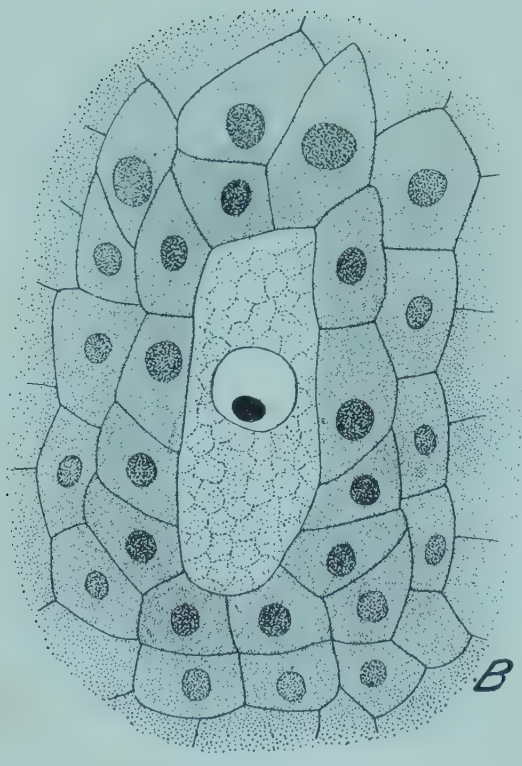

B

PLATE 26

A.—Cross section of the megaspore mother cell. × 800.
B.—Longitudinal section of the megaspore mother cell. × 800.
C.—Longitudinal section of the 2-celled embryo sac. × 800.

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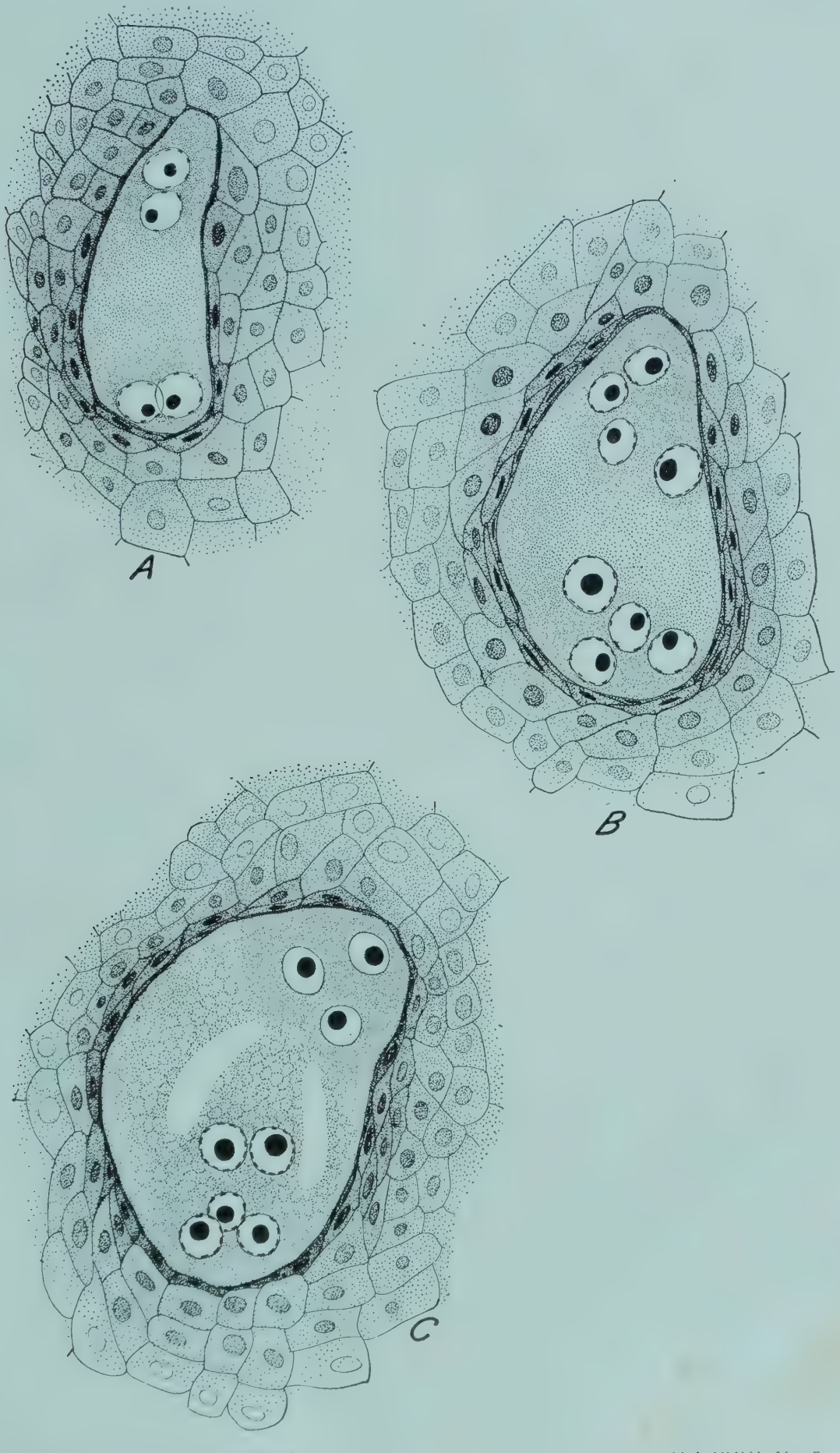
PLATE 27

A.—Longitudinal section of the 4-celled embryo sac. × 800.

B.—Longitudinal section of an 8-celled embryo sac at the time the polar nuclei have started to migrate. × 800.

C.—Longitudinal section of an 8-celled embryo sac after the polar nuclei have migrated. × 800.


A
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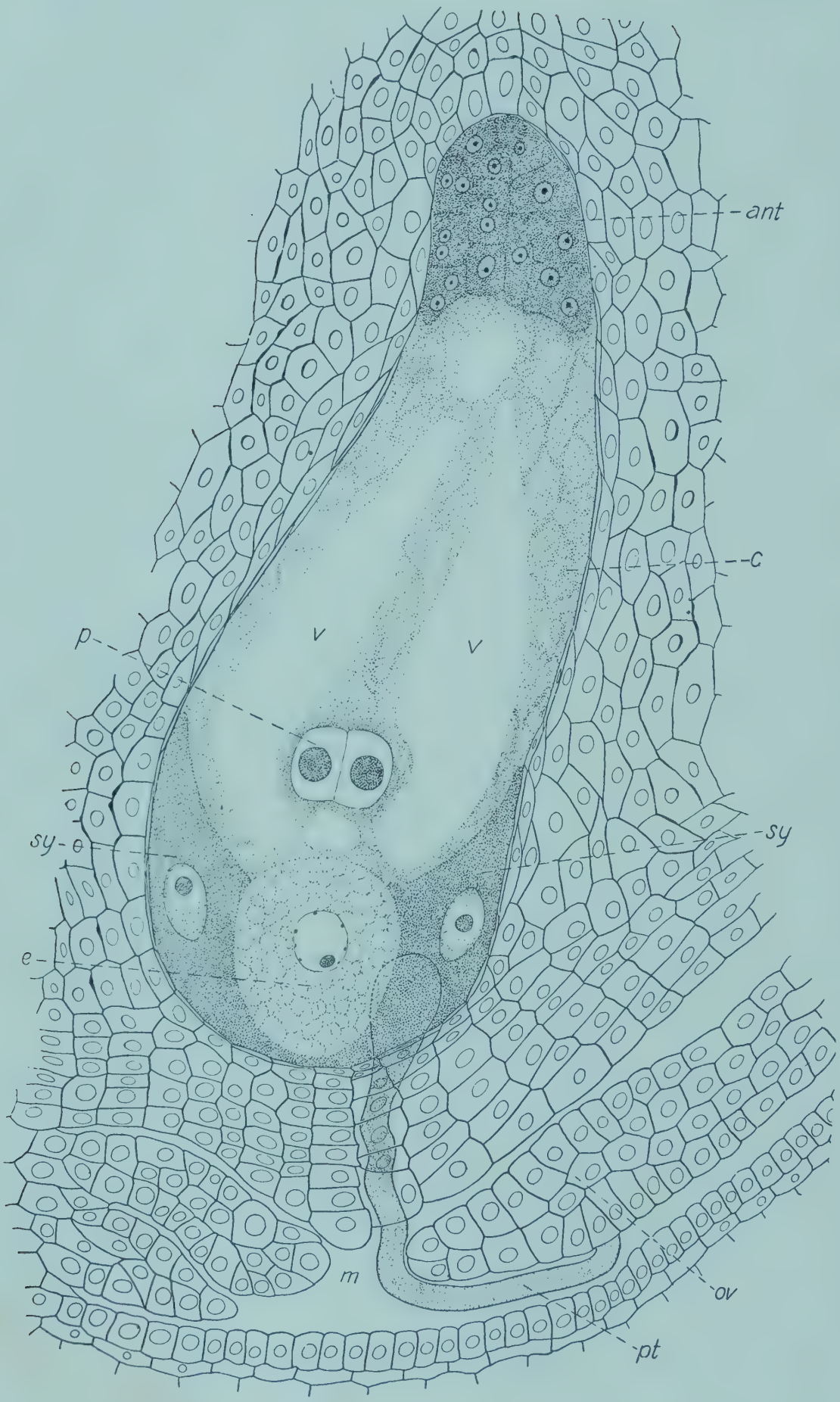

ant
c
p
v
v
sy
sy
θ
e
m
ov
pt

PLATE 28

Longitudinal section of a mature embryo sac just previous to fertilization: e, egg; sy, synergids; p, polars; ant, antipodals; pt, pollen tube; m, micropyle; v, vacuole; c, cytoplasm; ov, ovule coat. × 520.

PLATE 29

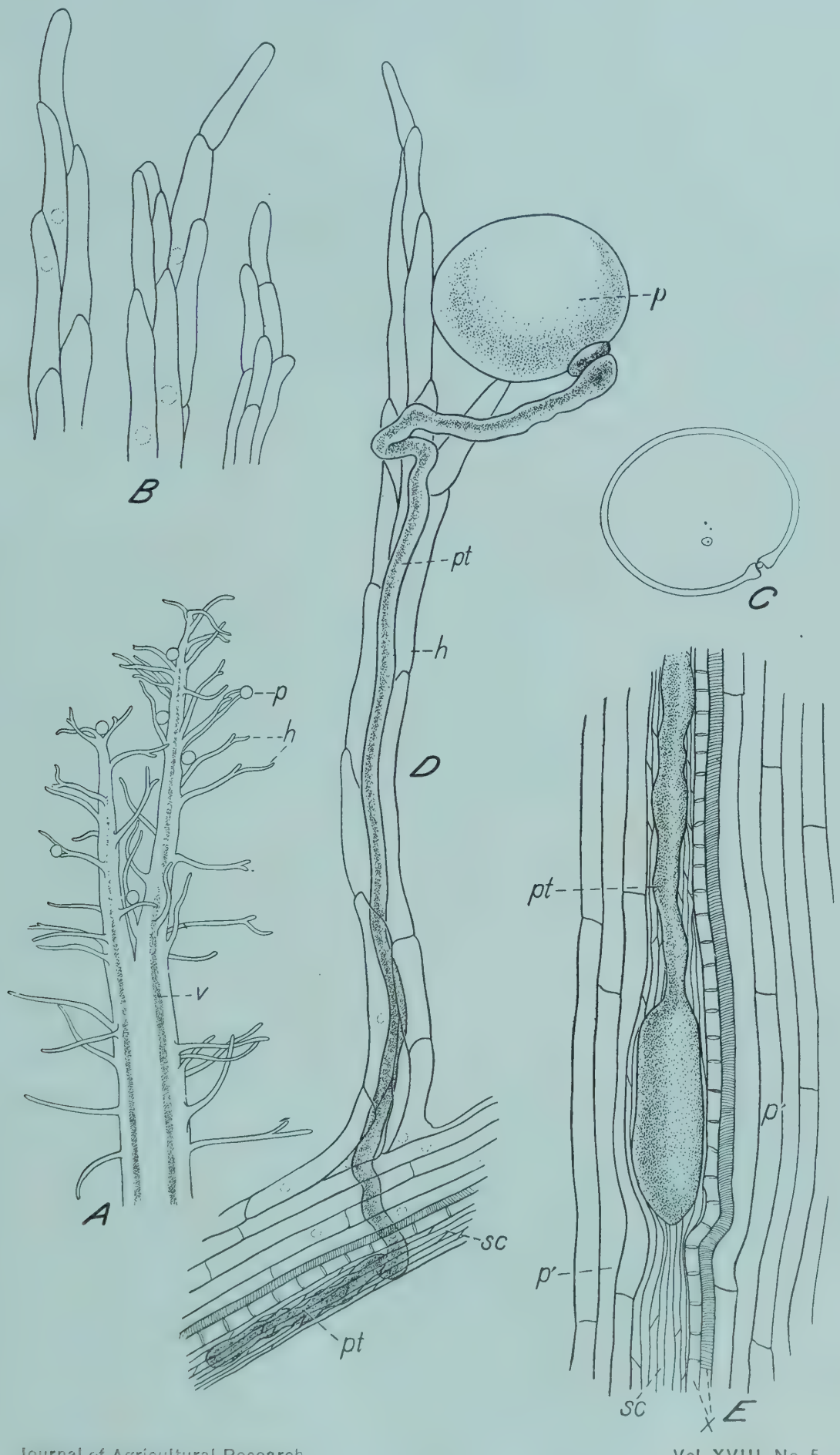
A.—End of a silk: p, pollen grains; v, fibro-vascular bundles; h, hairs. × 35.

B.—Tips of the hairs of the silk. It is on these hairs that most of the pollen grains lodge. × 250.

C.—Section of a pollen grain showing the germ pore and the relative size of the vegetative and sperm nuclei. × 250.

D.—Single hair of the silk, showing the general manner in which the pollen tube penetrates the sheath cells of the fibro-vascular bundle of the silk: p, pollen grain; h, hair; pt, pollen tube; sc, sheath cells of the fibro-vascular bundle. × 250.

E.—Longitudinal section of a fibro-vascular bundle of a silk, showing the position of the pollen tube as it grows down the silk: sc, sheath cells; x, xylem elements; p, parenchyma cells of the silk; pt, pollen tube, showing the enlarged tip. × 250.


B
p
C
pt
h
D
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p'
p'
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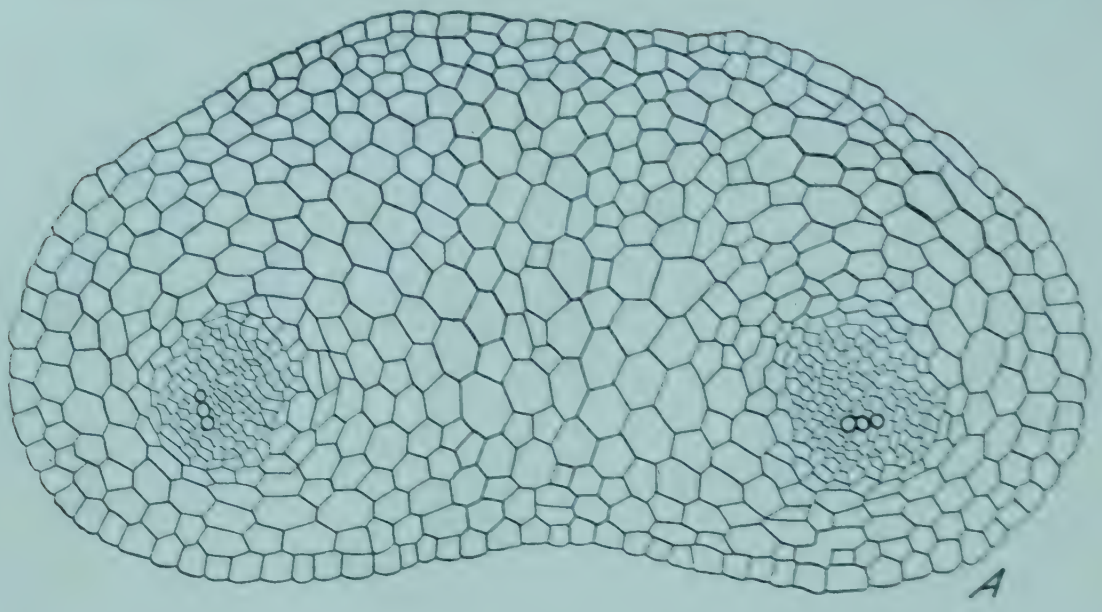
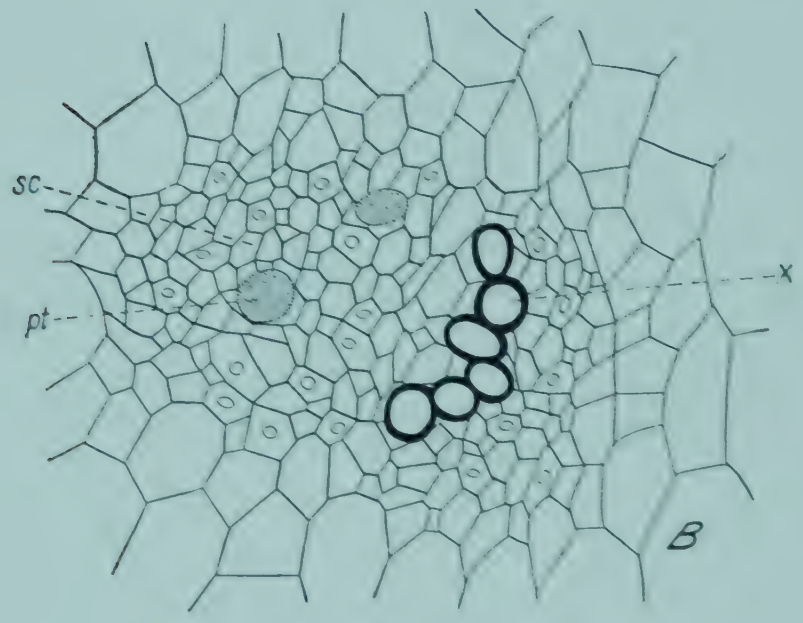
E


A


sc
pt
x
B

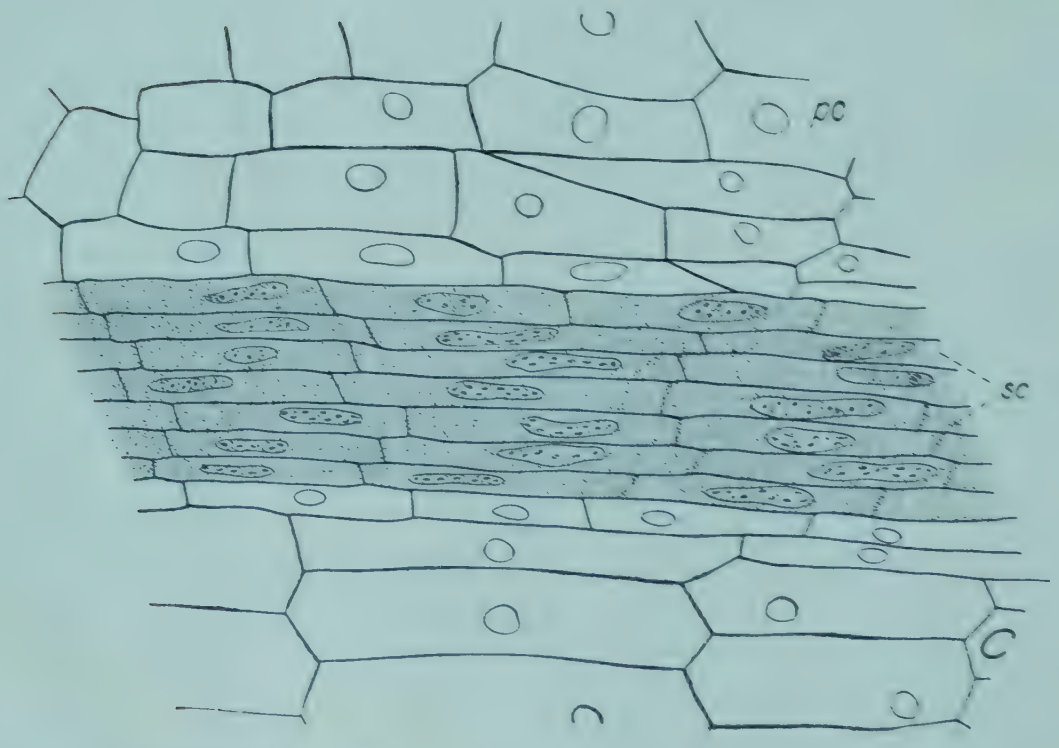

pc
sc
C

PLATE 30

A.—Cross section of a silk near its base, showing the position of the fibro-vascular bundles. × 220.

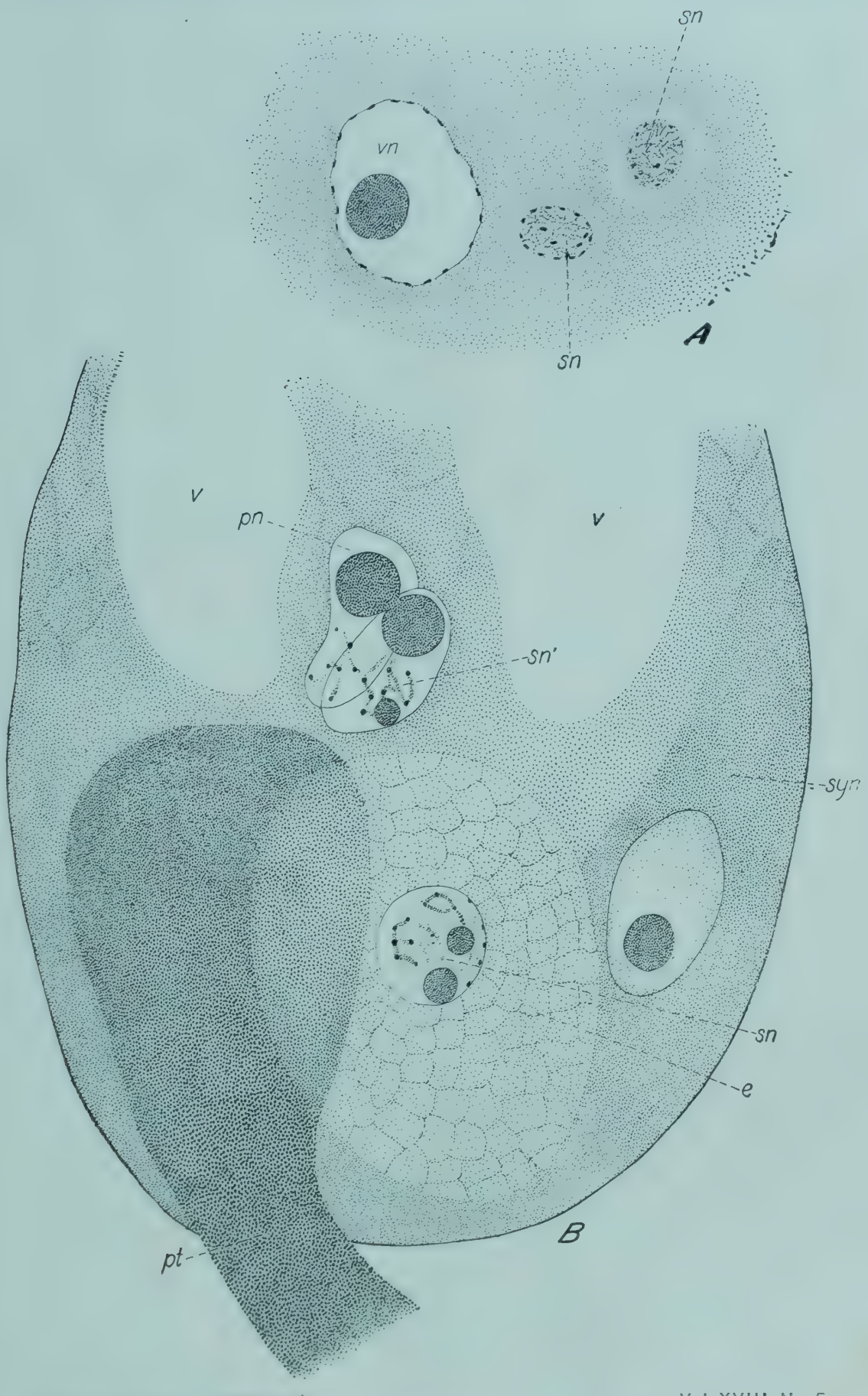
B.—Cross section of a fibro-vascular bundle of the silk: x, xylem elements; sc, sheath cells; pt, pollen tube. × 650.

C.—Longitudinal section through the sheath cells of the fibro-vascular bundles: sc, sheath cells; pc, parenchyma cells of the silk. It is between the sheath cells that the pollen tube works its way down the silk. × 650.

PLATE 31

A.—Vegetative and sperm nuclei of the pollen grain: vn, vegetative nucleus; sn, sperm nuclei. × 1,100.

B.—Longitudinal section of the lower portion of the embryo sac at the time of fertilization, reconstructed from two sections: pn, polar nuclei fusing; sn′, sperm nucleus fusing with a polar nucleus; e, egg; sn, sperm nucleus in the egg; pt, pollen tube; syn, synergid; v, vacuole. × 1,100.


sn
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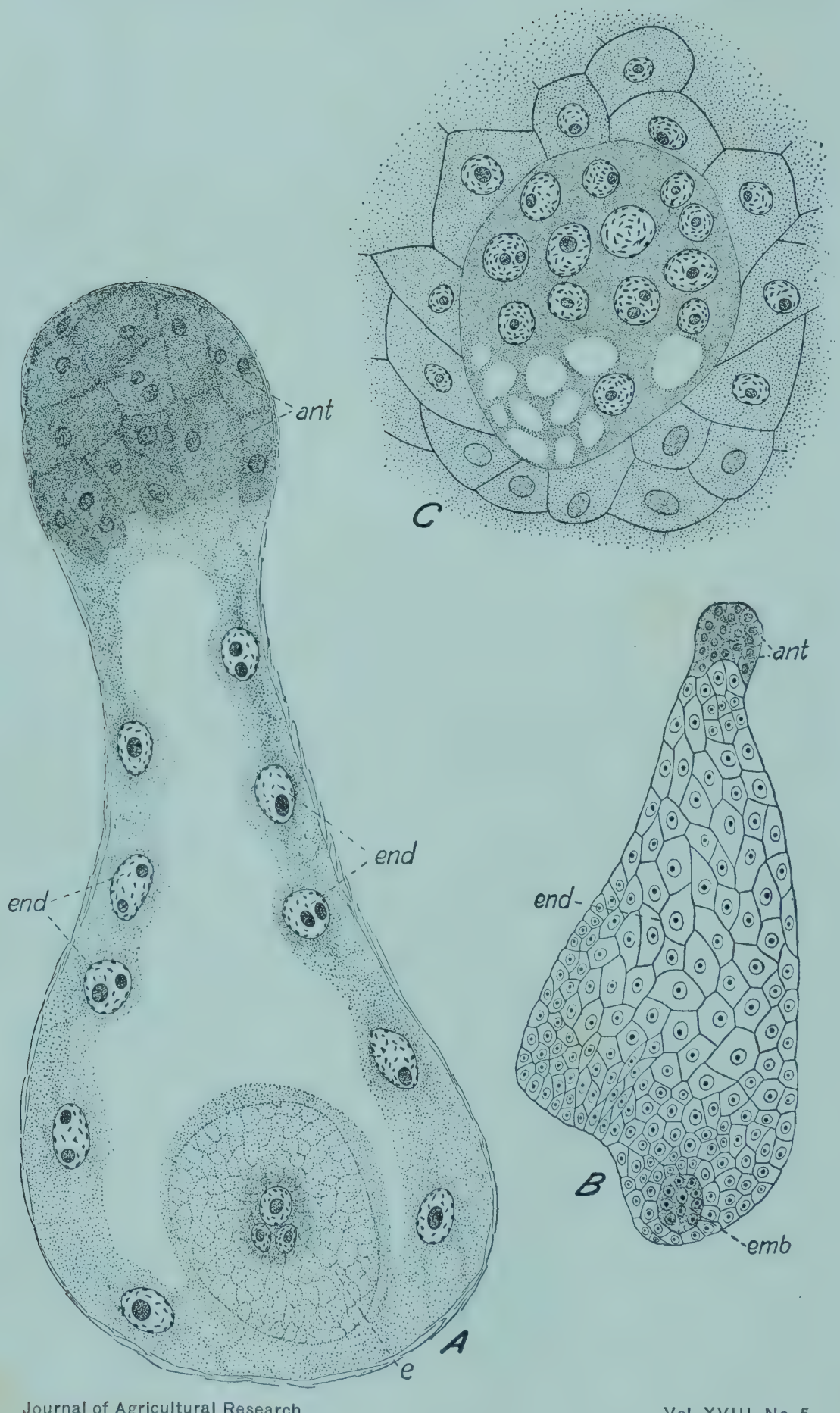
ant
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C
ant
end
B
emb
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e

PLATE 32

A.—Longitudinal section of the embryo sac 12 hours after fertilization: end, endosperm nuclei; e, egg in which one of the daughter nuclei has already divided; ant, antipodals. × 520.

B.—Longitudinal section of the embryo sac 36 hours after fertilization: end, endosperm; emb, embryo; ant, antipodal tissue. × 110.

C.—Longitudinal section of the young embryo at the stage shown in B. × 800.

RESPONSE OF CITRUS SEEDLINGS IN WATER CULTURES TO SALTS AND ORGANIC EXTRACTS

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INTRODUCTION

This paper deals with the growth of citrus seedlings in water cultures and their reaction to various salts and to organic matter in solution. The experiments were conducted mainly at Riverside, Calif., in connection with other investigations relating to the causes of the malnutrition of citrus trees. Lemon, grapefruit, and several varieties of orange seedlings (Blood, Tahiti, Mission, Valencia, Tangerine) were used in the experiments; but the work was confined largely to grapefruit and lemon on account of the relative ease with which the seeds could be secured.

EXPERIMENTAL METHOD

The seeds were sprouted in trays of coarse sand about 10 cm. deep and were covered to a depth of about 1 cm. At ordinary spring and summer temperatures it requires about 30 days to develop citrus seedlings to a stage suitable for culture work, but under greenhouse conditions this time can be reduced to about 20 days. The germination period of the grapefruit and lemon is somewhat less than that of the orange. The seeds should not be given much water during the germinating period. The citrus seedling is hardy and will stand drouth. Hot sunshine is likely to scald the primary leaves, so it is advisable to keep the seedlings in half shade.

When the seedlings were large enough to be used, the sand around the roots was saturated with water and the seedlings were withdrawn from the seed bed. The sand was washed off and the roots dipped into a thick paste of carbon black to a depth of about 1 cm. The carbon black sticks to the radicle and forms a well-defined index, so that the subsequent elongation of the root tip can be accurately measured. Five to 10 seedlings bound together lightly with a small piece of absorbent cotton were usually placed in each culture flask. Of the citrus seedlings so far tested, the lemon is by far the most satisfactory for use in water cultures, for the radicle is straight and the cotyledons do not separate as in the grapefruit.

It was found that the citrus seedling is especially sensitive to the toxic substances in distilled water. A treatment with carbon black that would

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purify the water sufficiently for wheat seedlings was inadequate for the citrus seedling. It was necessary to use an extra large amount of carbon black or to bring the water to the boiling point in the presence of carbon black and filter in order to obtain water in which citrus seedlings would grow. Individual variation in the resistance of the seedlings was frequently observed. In a toxic solution that would kill nine of the seedlings, the tenth would sometimes grow vigorously.

ORGANIC EXTRACTS

In the early experiments organic extracts from acid upland peat (leaf mold) and horse manure were employed. The organic matter was first extracted to obtain the water-soluble portion and then extracted with 3 per cent ammonia. These extracts were filtered and evaporated separately to dryness and the dried material used to make up standard organic solutions. The peat extract proved to be fully as satisfactory for cultures as that from manure and has been employed in most of the work.[1]

The effect of organic matter upon the growth of the lemon seedling is shown in Table I. The result in each instance represents the total root growth of 10 seedlings in six days. It will be seen that the water-soluble extracts of peat in concentrations of 10 parts per million or more produced root elongation double that produced with cultures in carbon-treated water.

TABLE I.—*Effect of peat extract on root elongation of citrus seedlings*

Treatment.	Root growth.
	Mm.
Carbon-treated water, control	90
Carbon-treated water, plus 5 p. p. m. water-soluble peat	115
Carbon-treated water, plus 10 p. p. m. water-soluble peat	197
Carbon-treated water, plus 50 p. p. m. water-soluble peat	197
Carbon-treated water, plus 100 p. p. m. water-soluble peat	236
Carbon-treated water, plus 500 p. p. m. water-soluble peat	178
Carbon-treated water, plus 1,000 p. p. m. water-soluble peat	190

A similar series of experiments with grapefruit seedings, carried on at the same time, gave results almost as marked. The root growth in the two cultures is illustrated in Plate 33, A, B.

The stimulating effect of the soluble organic matter in very low concentrations was verified by repeated tests and was observed not only for grapefruit and lemon seedlings but for Blood, St. Michael, Tahiti, Valencia, and Tangerine oranges as well. The ammonia extract of the peat, freed from ammonium hydrate (NH_4OH) by evaporating to dryness, was as effective as the water-soluble extract in stimulating the root growth of the seedlings.

[1] This peat is the same as that employed by Coville in his investigation of blueberry culture. (COVILLE, F. V. EXPERIMENTS IN BLUEBERRY CULTURE. U. S. Dept. Agr. Bur. Plant Indus. Bul. 193, 100 p., 31 figs., 18 pl. 1910.

The organic matter extracted from upland peat is decidedly acid, but the degree of acidity varies greatly in different samples. In the upland peat employed in this work, 1 gm. of the soluble material extracted with 3 per cent ammonium hydrate, after being evaporated to dryness to remove the ammonia and again dissolved in water free from carbon dioxid, required 0.088 gm. sodium hydroxid to neutralize it, using phenolphthalein as an indicator. The acidity is therefore about 8 per cent of an hydrochloric acid solution of the same concentration by weight. Since the root elongation was stimulated in the most concentrated organic extracts used (1,000 parts per million), it appears that at least in the presence of the other constituents of the peat extract the growth of the citrus seedlings is not inhibited by the organic acids present in concentrations equivalent to 80 parts per million hydrochloric acid.[1]

The figures in Table I, showing the root elongation, are not to be considered as representing accurately the relative stimulation of the various peat concentrations. Often a solution of 10 parts per million of peat would give as good plants as a solution of higher concentration. No attempt was made to find the amount of organic matter needed for maximum growth. Neither potassium chlorid nor sodium nitrate in water cultures stimulated the growth of the seedlings, so the action of the organic matter is not to be attributed to the addition of these nutrient salts.

STIMULATING ACTION OF CALCIUM CARBONATE AND BICARBONATE

Calcium carbonate was found to have as pronounced an effect as organic matter in stimulating the root elongation of citrus seedlings. This is shown in the following table, the figures representing the total root growth of 10 seedlings in eight days. About 0.1 gm. of calcium carbonate was added to each culture flask, which contained about 250 cc. of solution.

TABLE II.—*Stimulation of root growth of citrus seedlings by calcium carbonate*

Seedling.	Root growth in—	
	Carbon-treated water.	Carbon-treated water plus calcium carbonate.
	Mm.	*Mm.*
Lemon	92	435
Grapefruit	100	275

[1] The nature of the organic acids present in the upland peats of the United States does not appear to have been specifically investigated. Some of the organic acids and other substances of definite chemical composition which have been isolated from alkaline extracts of soils are listed in a paper by Shreiner and Shorey, who state that the vegetable acids of the hydroxy-fatty series which are added to the soil with the plant residues apparently soon break down into simpler compounds. (SCHREINER, Oswald, and SHOREY, Edmund C. CHEMICAL NATURE OF SOIL ORGANIC MATTER. U. S. Dept. Agr. Bur. Soils Bul. 74, p. 13. 1910.)

The stimulating action of calcium carbonate was observed with all the different kinds of seedlings studied. Its effect on lemon and grapefruit seedlings is illustrated in Plate 33, C–F.

Carbon dioxid was passed through carbon-treated water containing an excess of calcium carbonate. The resulting solution was found upon analysis to contain over 400 parts per million calcium as calcium bicarbonate. After portions were withdrawn and slightly agitated to expel the excess of carbon dioxid, citrus seedlings were introduced into the solution. With all the seedlings tried a stimulating effect was noticeable.

AMELIORATING ACTION OF LIME AND ORGANIC MATTER WHEN ADDED TO TOXIC SOLUTIONS

A slightly toxic solution was prepared by mixing one part of untreated distilled water with three parts carbon-treated water. This gave a solution that would stop the root development of all the seedlings. The addition of calcium carbonate (solid phase present) and organic matter to this solution invariably enabled the plant to overcome the toxic effect of the solution. Many tests of this kind were made with all kinds of citrus seedlings. A fair example of the relative root development of a 12-day-old culture of grapefruit seedlings is shown in Table III.

TABLE III.—*Protective action of lime and organic extracts*

Treatment.	Root development.
	Mm.
Toxic water, control	0
Toxic water, plus 20 p. p. m. organic matter	145
Toxic water, plus calcium carbonate (solid phase present)	148

Calcium is known to have a marked antagonism to the toxicity of some inorganic salts. The protective and stimulating action of the calcium carbonate is, however, in this instance not explainable on this basis. Citrus seedlings showed no growth in toxic water mixtures to which 10 parts per million of calcium sulphate or of calcium chlorid had been added. The solubility of calcium carbonate in distilled water is approximately 10 parts per million. From the fact that calcium chlorid and calcium sulphate in the same concentration showed no protective action it appears that it is not the calcium ion itself that gives rise to the antagonistic action on distilled water toxins. Furthermore, the calcium content of the organic dried peat extract was only 0.3 per cent, so that protective action of the peat in concentrations of 20 parts per million is not attributable to the calcium it contains. Though the nature of the protective action has not been determined, the adsorption of the toxins on the calcium carbonate particles present in the solid phase and by the colloidal organic constituents is suggested as a possible explanation.

TOXIC LIMITS OF ALKALINE SALTS

The toxicity of calcium hydrate, sodium hydrate, and sodium carbonate was determined for lemon and grapefruit seedlings which showed practically the same degree of resistance. The cultures in calcium hydrate solutions while under observation were kept in a large desiccator containing quicklime. A concentration of 25 parts per million of calcium hydrate or sodium hydrate gave stimulating results. When the concentration of calcium hydrate was increased to 50 parts per million, signs of distress were noted. Little growth took place at 80 parts per million, and at 100 parts per million growth practically ceased. Here a wide variation was noted among the seedlings. Occasionally a vigorous seedling would withstand a concentration of 120 parts per million of calcium hydrate. This was exceptional, however, and it may be said that the citrus seedling will seldom tolerate 100 parts per million of calcium hydrate.

With sodium hydrate a concentration of 25 parts per million stimulated the root growth, and concentrations up to 200 parts per million were maintained with little harmful effect upon the plant. With 250 parts per million the growth was slight, while with 275 to 300 parts per million growth practically ceased. With sodium carbonate it was necessary to increase the concentration to 550 or 600 parts per million in order to stop the growth of the seedlings. It is of interest to note that a solution of this concentration would, on account of hydrolysis, contain sodium hydroxid in approximately the same concentration as that representing the toxic limit of sodium hydroxid. In other words, it is the hydrolyzed portion of the sodium carbonate which mainly determines the toxicity.

Since calcium hydrate and sodium hydrate have nearly the same equimolecular weights, it follows that the hydroxyl concentration in the toxic calcium hydrate solution is only about one-third that in the toxic sodium hydrate solution. It is evident that the metallic ion is contributing also to the toxicity.

When organic matter (extracted from peat with ammonia) which is acid in reaction and stimulating to root growth is added in the proportion of 100 parts per million to a solution containing 400 parts per million of sodium carbonate, a toxic body is formed that will kill the root tip of the seedlings. (Pl. 34, A–D.) This is not true, however, with organic matter extracted from peat with water. This class of reactions appears to be of importance in connection with the toxicity of alkaline soils and will be made the subject of a later report.

TOXIC LIMITS OF ACIDS

The toxic properties of only one inorganic acid, phosphoric acid, were investigated. This acid stopped the root development at a concentration of 20 parts per million, with both the grapefruit and the lemon. Attention has already been called to the fact that active root elongation takes place in organic extracts from peat having an acidity equivalent to 80 parts per million of hydrochloric acid. In overripe lemons the seeds are likely to germinate inside the fruit and the plumule and radical be pushed through the skin. Since the juice of the lemon contains 7 or 8 per cent citric acid, it appears that under natural conditions the lemon seeds will sprout in a strongly acid medium.

TOXIC LIMITS OF NITRATES AND OF AMMONIUM SULPHATE

It is known that the continued use of sodium nitrate in relatively large amounts tends to produce mottling of the leaves of citrus tress. Culture tests were accordingly made to determine the toxicity of the nitrate salts together with that of ammonium sulphate, which is also used in citrus districts as a source of nitrogen. The results are given in the following table, with the toxic limits of the same salts in the presence of lime.

TABLE IV.—*Toxic limits of nitrates and ammonium sulphate for citrus seedlings*

Salt.	Toxic limit.
	P. p. m.
Sodium nitrate	1,800
Potassium nitrate	3,500
Calcium nitrate	10,000
Ammonium sulphate	1,000
Sodium nitrate and calcium carbonate (solid phase)	6,000
Ammonium sulphate and calcium carbonate (solid phase)	2,000

It will be seen that marked differences occur in the toxic limits of the various salts, sodium nitrate being five times as toxic as calcium nitrate. The toxic limits for this group of salts are so high that the matter may appear to be of no practical import. But a simple calculation will show that the surface feeding roots of citrus trees are at times subjected to fertilizer concentrations in field practice so great as to approach toxic conditions. Application of 2 to 3 pounds of nitrate of soda per tree, or 200 to 300 pounds per acre, which is not an unusual practice for some citrus growers, would correspond approximately to a concentration of 70 to 100 parts per million in the soil of the surface foot. The fertilizer, moreover, is ordinarily applied to the open ground between the tree rows—that is, to not more than one-half the total soil area. If the moisture content of the soil were reduced to 10 per cent of the weight of the soil, the concentration of the sodium nitrate in the soil solution would range

from 1,400 to 2,000 parts per million—that is, it would approach the toxic limit. The surface crusts in citrus groves are often highly toxic to citrus seedlings.

However, mixed salts in the soil are not as a rule so toxic as the individual components. The protective or antagonistic action of calcium, for example, when added to toxic solutions of many inorganic salts is well known. Kearney and Cameron [1] found that gypsum and lime greatly increased the tolerance of white lupine and alfalfa seedlings for most of the salts found in saline soils. Osterhout has observed antagonism in many mixtures of salts and has shown that, in general, if one salt increases and the other decreases the permeability of the protoplasm, as determined by electrical conductivity, then the two salts tend to be antagonistic.[2] He has also found that while none of the monovalent ions, excepting hydrogen, decrease permeability, all the bivalent ions tested (calcium, magnesium, barium, etc.) do so to a marked degree.[3] In other words, the two groups are antagonistic.

It was consequently to be expected that calcium carbonate would increase the tolerance of citrus seedlings for sodium nitrate or potassium nitrate. The effect was most pronounced with a mixture of sodium nitrate and calcium carbonate (solid phase present) in which a concentration of 6,000 parts per million of sodium nitrate, or over three times that with sodium nitrate alone, was reached before the death of the root tip occurred (Pl. 34, E–G). With ammonium sulphate the death limit in the presence of calcium carbonate was reached at a concentration of about 2,000 parts per million, or twice that with ammonium sulphate alone. The filtrate obtained after shaking the higher concentration of sodium nitrate with calcium carbonate in excess and removing the solid phase produced as good plants as when the solid phase was present. The mechanical or adsorptive action of the latter, therefore, has no effect. The addition of small quantities of quartz flour, carbon black, or organic matter to the solution was also without effect on the toxic limit of the nitrate solutions.

When calcium nitrate or calcium sulphate in concentrations equivalent to the saturation concentration of calcium carbonate in water (10 parts per million) was added to sodium nitrate solutions, the toxic limit was not raised appreciably above that of sodium nitrate alone. As the concentrations were increased, the protective action developed; and in concentrations of 100 parts per million calcium chlorid or calcium sulphate was about as effective as calcium carbonate in raising the toxic limit of sodium nitrate. On account of the reaction between sodium nitrate and

[1] KEARNEY, Thomas H., and CAMERON, Frank K. EFFECT UPON SEEDLING PLANTS OF CERTAIN COMPONENTS OF ALKALI SOILS. *In* U. S. Dept. Agr. Rpt. 71, p. 7–60. 1902.

[2] OSTERHOUT, W. J. V. ON THE NATURE OF ANTAGONISM. *In* Science, n. s. v. 41, no. 1050, p. 255–256. 1914.

[3] OSTERHOUT, W. J. V. ON THE DECREASE OF PERMEABILITY DUE TO CERTAIN BIVALENT RATIONS. *In* Bot. Gaz., v. 59, no. 4, p. 317–330, 11 fig. 1915.

calcium acrbonate when the latter salt is present in the solid phase, the calcium-ion concentration is considerably increased above that of a saturated solution of calcium carbonate alone. This increase in the concentration of the calcium ion, which gives the protective action, is probably the explanation of the failure of calcium chlorid and calcium sulphate to exert a protective action at concentrations corresponding to the solubility of calcium carbonate alone. Calcium nitrate and calcium hydrate in small amounts were also as effective as calcium carbonate. Organic matter had no ameliorating effect on the toxic action of sodium nitrate.

SUMMARY

Seedlings of various citrus stocks, including lemon, grapefruit, and several varieties of sweet oranges, showed no characteristic differences in response in water cultures or in resistance to toxic solutions.

Very dilute organic extracts from upland peat (10 parts per million or more) produced a marked stimulation of the root growth of citrus seedlings. Corresponding concentrations of sodium nitrate or potassium chlorid did not stimulate the root development.

Calcium carbonate stimulated the root growth and exerted a pronounced antagonistic action to toxic solutions of nitrates and ammonium sulphate.

Peat extract in very dilute concentrations (20 parts per million) and calcium carbonate (solid phase present) both protected citrus seedlings to a marked degree against the toxins of distilled water.

The tolerance of citrus seedlings for alkaline salts is relatively high. The toxic limit for calcium hydrate was 100 to 120 parts per million, for sodium hydrate 250 to 300 parts per million, and for sodium carbonate 550 to 600 parts per million. The hydroxyl concentration in the toxic calcium hydrate solution is only about one-third that of the toxic sodium hydrate solution.

When soluble organic matter which is acid in reaction and stimulating to citrus seedlings in concentrations up to 1,000 parts per million or more is added to a sodium carbonate solution of 400 parts per million which in itself is not toxic, a highly toxic solution is formed which will kill the root tips of citrus seedlings. This reaction appears to be of importance in connection with the toxicity of soils containing small amounts of sodium carbonate.

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PLATE 33

A.—Grapefruit roots, 12 days old, grown in distilled water.

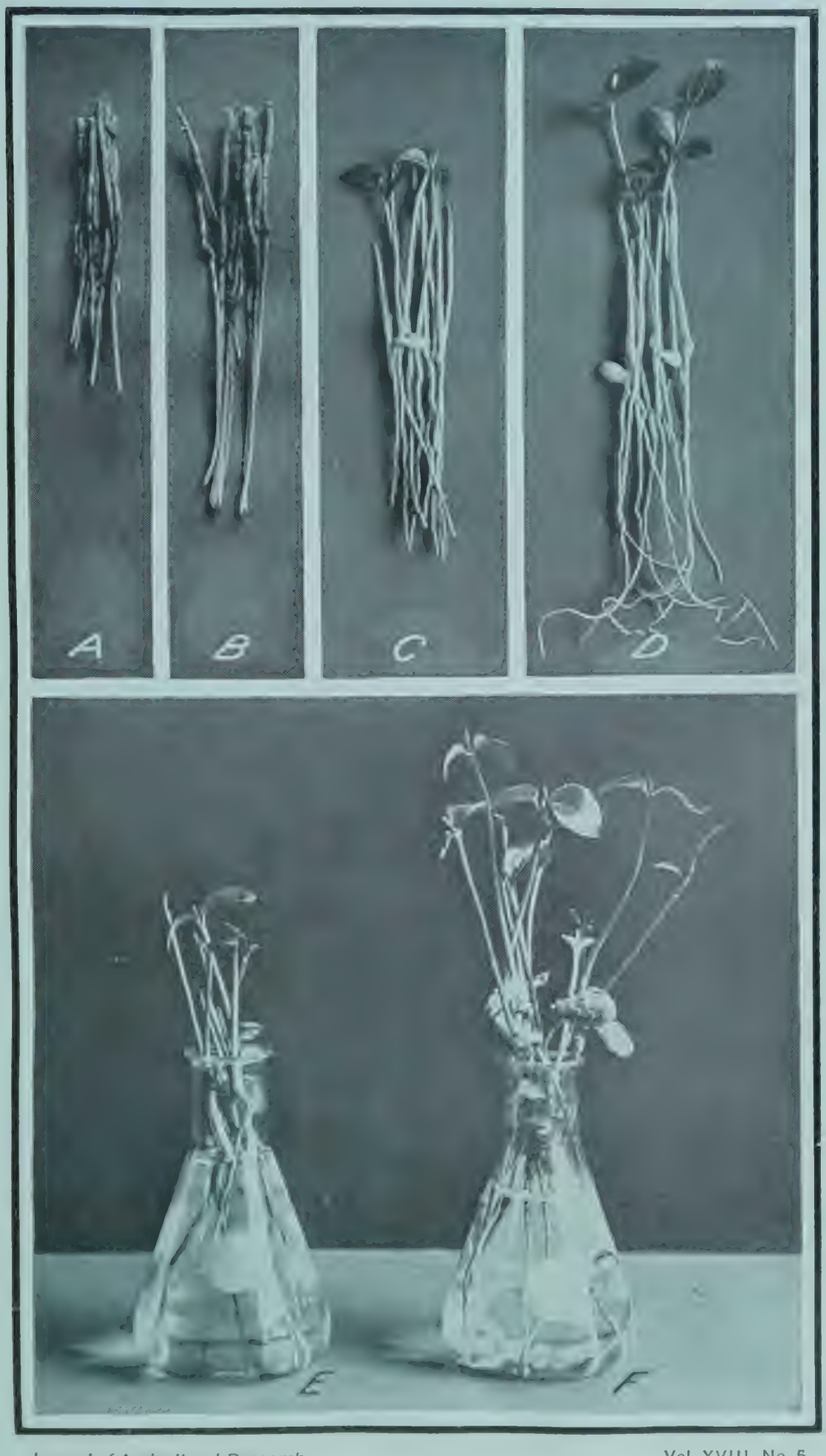
B.—Grapefruit roots, 12 days old, grown in distilled water plus 100 parts per million water-soluble peat.

C.—Lemon seedlings, 21 days old, grown in carbon-treated distilled water.

D.—Lemon seedlings, 21 days old, grown in carbon-treated distilled water plus calcium carbonate.

E.—Grapefruit seedlings, 20 days old, grown in carbon-treated water.

F.—Grapefruit seedlings, 20 days old, grown in carbon-treated water plus calcium carbonate.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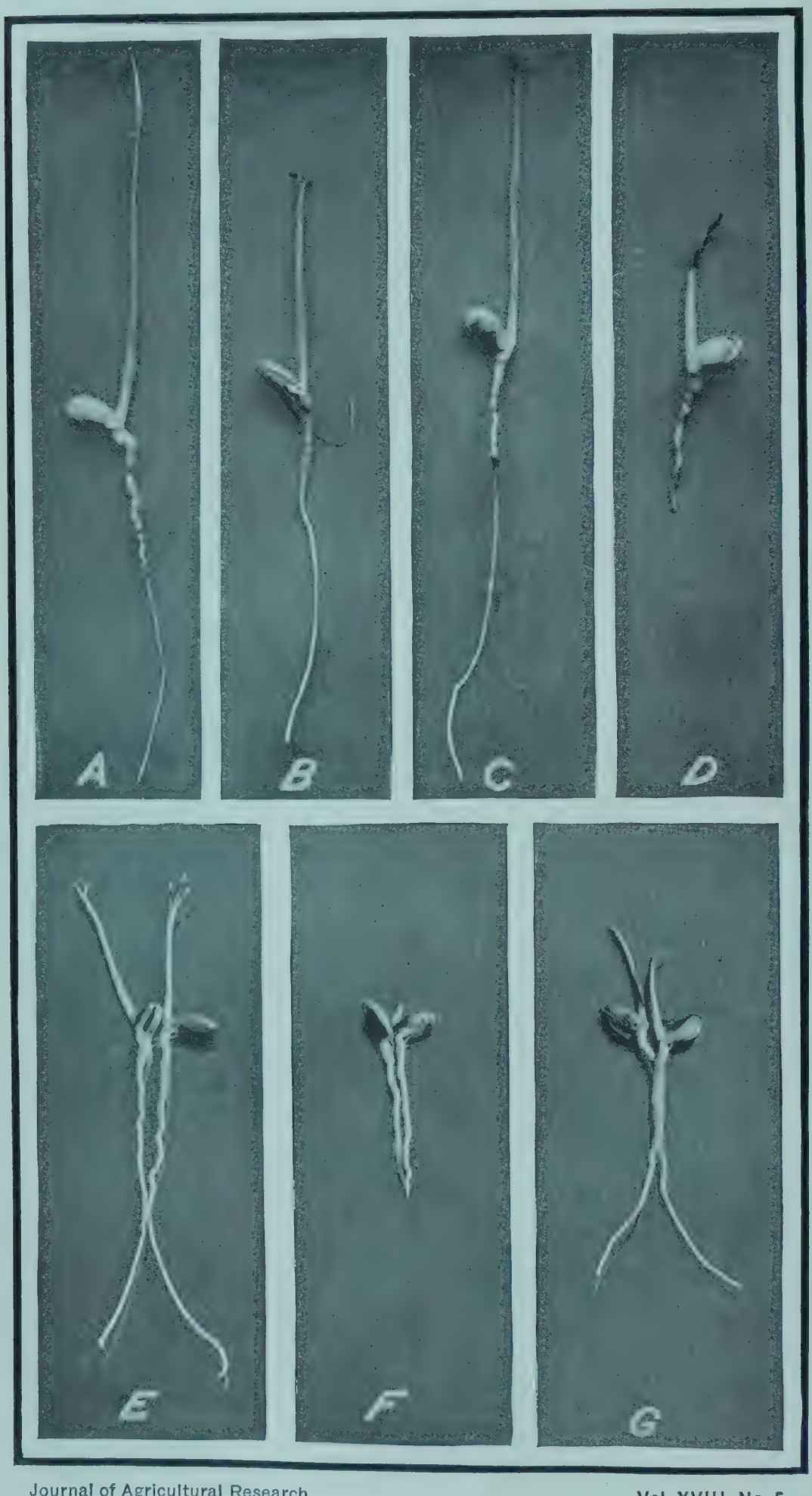
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PLATE 34

A.—Lemon seedlings, 16 days old, grown in distilled water as control.

B.—Lemon seedlings, 16 days old, grown in 400 parts per million sodium carbonate.

C.—Lemon seedlings, 16 days old, grown in 100 parts per million ammonia-soluble humus.

D.—Lemon seedlings, 16 days old, grown in 100 parts per million ammonia-soluble humus plus 400 parts per million sodium carbonate.

E.—Citrus seedlings grown in carbon-treated water.

F.—Citrus seedlings grown in 4/10 per cent sodium nitrate ($NaNO_3$).

G.—Citrus seedlings grown in 4/10 per cent sodium nitrate with calcium carbonate ($CaCO_3$).

PHYSIOLOGICAL STUDY OF THE PARASITISM OF PYTHIUM DEBARYANUM HESSE ON THE POTATO TUBER

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INTRODUCTION

The physiology of parasitism and the relations existing between the host and parasite have been the subject of numerous investigations, many of which have taken up the method by which the fungus obtains entrance into the plant or passes from cell to cell within the tissues of its host.

There are, of course, several possible ways by which a parasitic plant may obtain entrance into the cells of its host plant. It may enter through an opening already made; if it makes the opening itself, it may push its way through mechanically, or it may soften or digest the cell walls. It is possible, also, that the fungus hyphae might so stimulate the cells of the host plant that enzyms secreted by the host itself would break down its own cell walls and allow the fungus to enter. A combination of these methods is, of course, possible—for example, a fungus might penetrate the cell wall by a small opening and then enlarge this opening either mechanically or by a solution of a portion of the cell wall. Some of the investigations on this subject will be considered here.

In 1886 De Bary (*3*)[1] showed that *Sclerotinia libertiania* secreted a toxic substance which killed the cells ahead of the growth of the fungus. He concluded that the breaking down of the cell walls was due to an enzym secreted by the fungus.

Ward (*27*) concludes, in his study of Botrytis on lily, that the tip of the fungus hypha "excretes relatively large quantities of ferment substance and dissolves its way into the cell wall." Nordhausen (*21*) considers that *Botrytis cinerea* dissolves its way through the cell walls of its host plant. Büsgen (*9*) considers that this fungus does not make its way through cell walls or even cuticle by mechanical means alone. Miyoshi (*20*) showed that *Botrytis cinerea* could force its way through membranes of collodion, paper, and other substances, and details some experiments in which Penicillium pushed its way through gold leaf. Peirce (*23*) has shown that the haustoria of Cuscuta will puncture tinfoil 0.2 mm. in thickness.

[1] Reference is made by number (italic) to "Literature cited," p. 295-297.

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Brown (*6*) in a recent study of parasitism in Botrytis has shown that this fungus secretes an enzym which breaks down the middle lamellae of tissues which it invades. He demonstrated that the enzym secretion was more powerful from freshly germinated spores than from old cultures. A toxin, which is apparently closely associated with the enzym, is also secreted. This toxin is not oxalic acid or an oxalate. Blackman and Welsford (*5*), in the second of this series of papers, have shown that this fungus apparently penetrates the cuticle of the broad bean leaf by pushing its way through mechanically. These writers are in agreement with Brown that an enzym secreted by the fungus breaks down the tissues in the interior of the leaves. Brown (*7*) later showed that the infecting germ tubes were unable to affect chemically the cuticle of the host plant and that the toxic substance could not pass through the cuticle. He concludes that penetration of the cuticle must take place in a purely mechanical way. In the fourth paper of this series (*8*) he contrasts thick and thin sowings of spores and finds that thick sowings 2 to 4 days old yield the most active preparations of enzym. He considers that the cytase is much more active at the tip of the hypha.

From this review, which of course covers only part of the literature on this subject, it is apparent that there is good evidence that some parasitic plants make their way into their host plants by breaking through the tissues mechanically; but there is no doubt that some fungi secrete enzyms which break down the cell walls of certain plants and are thus able to make their way through the tissues of their hosts.

The parasitism of *Pythium debaryanum* Hesse on some of its numerous hosts has been investigated, but how it gains entrance into the host plant seems not to have received any considerable attention. This fungus has recently been shown to be the cause (*15*) of a tuber-rot of potatoes which is of considerable commercial importance in the San Joaquin Valley of California. A method of controlling this disease under commercial conditions has been worked out and described (*17*). In the present study the effect of the fungus on the sugars, pentosans, and starch of the potato tuber was determined, and the rate of growth of the fungus in three different varieties of potatoes was measured. An attempt was made to correlate certain physical and chemical characteristics of the potatoes with their susceptibility or resistance to this disease, and the growth of the fungus in the potato tissue was observed and studied. Some information on the mode of entrance of this fungus into the cells of the potato was obtained, and a possible explanation was found as to why some varieties of potatoes are much more susceptible to this disease than others.

EXPERIMENTAL WORK

The methods followed in the study of the effect of the fungus on the starch, sugars, and pentosans of the potato tuber were essentially those described for the work with the Fusarium tuber-rots (*16*). They will not be discussed here. The results of the analyses of the sound and rotted quarters are shown in Table I.

TABLE I.—*Starch, sugar, and pentosan content of the sound and rotted quarters of potatoes rotted with Pythium debaryanum*

[Expressed as percentage of wet weight]

Pentosans.			Starch.			Sugar.				
						Sucrose as dextrose.			Reducing sugar as dextrose.	
Tuber No.	Sound quarter.	Rotted quarter.	Tuber No.	Sound quarter.	Rotted quarter.	Tuber No.	Sound quarter.	Rotted quarter.	Sound quarter.	Rotted quarter.
1.....	0.548	0.44	5....	16.07	14.64	9...	0.98	0.030	0.220	0.003
2.....	.39	.32	6....	16.61	16.34	6...	.63	.009	.252	.002
3.....	.33	.27	7....	16.73	13.04	10...	.11	.008	.392	.012
4.....	.35	.31	8....	15.65	11.42	11...	.46	.020	.440	.001

In Table I it is noticeable that the sugars, both sucrose and reducing sugars, had almost disappeared in the rotted portions of the tuber, while appreciable amounts were present in all the uninfected quarters. The fungus is evidently able to utilize the sugars of its host. In this its action is similar to that of *Fusarium oxysporium*, *F. radicicola*, and *F. coeruleum* (*13*) on potatoes, *Sclerotinia fructigenia* on apples (*4*) and peaches (*14*), *Sphaeropsis malorum* on apples (*12*), and *Rhizopus nigricans* (*25*) on strawberries, all of which cause a decrease in the sugar content of the host plant or part of the host invaded. The results with these several fungi seem to justify the conclusion that rot-producing fungi are usually able to break down and utilize the sugars of the host.

The starch content of the potato also decreases when rotted by *Pythium debaryanum*, as is shown in columns 5 and 6 of Table I. Starch grains were frequently found corroded; and an extract of the fungus mycelium, which had been grown on either potato plugs or mashed potatoes which had been sterilized, was able to pit potato starch grains as well as to digest gelatinized potato starch. In this respect the rot produced by this fungus is different from that produced by any of the three species of Fusarium mentioned above. With the Fusarium-rots no corrosion of the starch grains was noticeable, and the starch content of the rotted portions was not lower than that of the corresponding sound quarter. An extract of the mycelium of any of these three fungi was apparently incapable of corroding grains of potato starch even

after a long period, though gelatinized potato starch and soluble starch were readily digested by the extracts. The results obtained with *P. debaryanum* on potato starch are not in accord with the findings of Ward (*26*), who concluded that this fungus did not attack the starch of the potato. His conclusions were based entirely on microscopical observations. The pentosan content of portions of the tuber-rots by *P. debaryanum* is somewhat lower than that of the corresponding sound quarters, and from this it may be concluded that the fungus is able to digest the pentosans. This conclusion is supported by the fact that in potatoes rotted by this organism the middle lamellae of the cells are broken down and the cells may be readily teased apart on a slide. Extracts of the mycelium also digest the middle lamellae, and a slice of potato ½ mm. in thickness disintegrates in 12 hours when immersed in it. The middle lamellae, however, seem to be the only portion of the cell wall affected, for when a bit of rotted tuber is placed on a slide and teased out the cells float free, while only in exceptional cases are broken cells seen. The fungus penetrates the tissue in all directions but seems most frequently to pass directly through the cell wall.

In inoculation experiments with this fungus it was found that Bliss Triumph and Green Mountain potatoes were very susceptible to this disease, while the White McCormick potatoes were not. In the experiments all the Bliss Triumph and Green Mountain tubers rotted eventually—90 per cent as a result of the first inoculation—while with the McCormicks only about 30 per cent of the potatoes seemed to be susceptible to the disease even when inoculated three different times. When a McCormick tuber did become infected, the rot usually developed very slowly. This variety, while not immune to the disease, seemed to be highly resistant.

Measurements of the rate of growth of the fungus were made in tubers of the three different varieties mentioned. The method followed was to cut cylinders of the potato tubers about 12 mm. in diameter and 30 mm. long. These cylinders were placed on end in a small moist chamber and inoculated on the upper end from stock cultures of the fungus. After incubating for 24 hours the cylinders were sliced transversely into sections 1 mm. in thickness. These slices were numbered in order and placed in a moist chamber. The slices in which the rot developed were noted, and it was thus possible to determine within a millimeter the distance the fungus had progressed in 24 hours. This method is somewhat similar to the method followed by Jones, Giddings, and Lutman (*18*) in their study of the resistance of potatoes to *Phytophthora infestans*. The rapid rate of growth of the fungus used in the present study, however, made it possible to simplify the method considerably. The results of these experiments are shown in Table II.

TABLE II.—*Average rate of growth of Pythium debaryanum in tissue of Bliss Triumph, Green Mountain, and McCormick potatoes*

Experiment No.	Green Mountain.		Bliss Triumph.		McCormick.	
	Number of cylinders.	Average growth per hour.	Number of cylinders.	Average growth per hour.	Number of cylinders.	Average growth per hour.
		Mm.		*Mm.*		*Mm.*
1	6	0.354	7	0.430	7	0.071
2	9	.458	9	.425	8	.187
3	7	.290	8	.453	8	.049
Average		.366		.436		.102

It is noticeable in Table II that the rate of growth of the fungus in Bliss Triumph and Green Mountain tubers is from three to four times as rapid as in McCormick under the conditions of the experiment. In some cases the fungus was apparently unable to affect the cylinders from the McCormick and had not, at the end of the experiment, penetrated into the first millimeter of tissue. Other cylinders from this variety were much more susceptible, however, so that the average rate of growth of the fungus, as shown in the table, is fairly high.

In order to relate this rate of growth to the number of cells traversed, measurements of the cells in the cortex and central portions of tubers of these three varieties were made. About 1,500 measurements were made with each variety. The averages for the cortex and central portions of the three varieties are given below:

TABLE III.—*Average size of cells in the three varieties of potatoes from 1,500 measurements on each variety*

	Bliss Triumph.	Green Mountain.	McCormick.
Cortex	269μ × 303μ	294μ × 311μ	269μ × 303μ
Central portion	318μ	318μ	347μ

If it is considered, then, that the same rate of growth holds for the cortex and interior of the tuber, the average length of time required for the fungus to pass through an average cell in the interior would be 43, 50, and 204 minutes, respectively, for the three varieties, Bliss Triumph, Green Mountain,[2] and McCormick. The fact that the cells are so nearly of the same size in the three varieties would eliminate the possibility that the relatively slow rate of growth of the fungus in the McCormick tubers was due to the small size of the cells and the consequently larger number

[2] In a former paper (17) one of the writers gives the size of the cell in a Green Mountain tuber as 138.7μ, which is erroneous. The tuber mentioned was of the Burbank variety. The size of the cells and rate of growth given in the present paper are correct for this variety.

of cell walls for the fungus to penetrate in traveling a given distance. It may, however, be due to some resistant quality of the cell wall.

That the fungus secretes a toxic substance which kills potato cells was demonstrated experimentally by a method somewhat similar to that followed by Brown (*6*, *7*, *8*) in his work with *Botrytis cinerea*. Cultures of the fungus were grown for two weeks on sterilized potato mush, and potato plugs and the mycelium were removed in such a way that none of the culture medium adhered to the mat of mycelium. The mycelium was then ground in a mortar with sand, extracted with distilled water, and filtered. Cylinders about 1 cm. in diameter were cut from potato tubers and sliced into disks 0.5 mm. in thickness. Some of these disks were placed in the extract of mycelium and some in distilled water and examined at intervals. After three hours the disks in the fungus extract had lost their turgidity so that when grasped by the edge with a pair of forceps and held in a horizontal position they collapsed limply. The disks from the distilled water preparation remained turgid for 12 hours or more. Disks from the preparation of fungus extract did not resume their normal turgidity when washed and placed in distilled water. The cells of the potato are apparently killed by some substance extracted from the ground mycelium. The loss of turgidity can not be accounted for by a loss of water from the potato cells caused by a higher osmotic pressure in the extract, because tests showed that the lowering of the freezing point of the extract used was only about one-fifth that of the juice from the potato tuber. All three varieties of potatoes used in these experiments behaved in the same way. From these experiments it seems hardly probable that resistance to fungus attack can be due directly to the living protoplasm.

The macerating effect of this extract on the potato tissue has been mentioned earlier in this paper. The properties of the toxic substance secreted by the fungus were not determined, though the problem is well worthy of investigation.

It has been shown in some cases that resistance to certain fungus diseases was correlated with higher acidity of the host plant tissues. Thus Averna-Sacca (*2*) has shown that the resistance to diseases of grapes caused by Oidium and Peronospora was correlated with a relatively high acidity. Comes (*11*) has demonstrated that a variety of wheat (Rieti), resistant to rust, has an acidity considerably higher than the varieties in the same locality which are susceptible to this disease. Further, this writer has shown that when this resistant variety is grown in other localities where the environmental conditions tend to produce a plant of lower acidity, the plant is susceptible to the disease. These researches indicate that acidity may play a very important rôle in the resistance of a plant to disease.

There are, of course, many other factors that tend to influence the resistance or susceptibility of a plant to disease. The literature on this

subject has been ably reviewed in the papers of Ward (*28*), Appel (*1*), Orton (*22*), and Butler (*10*) and will not be considered in this paper except as it relates directly to the problem.

Inasmuch as *Pythium debaryanum* is rather susceptible to acids, it was considered worth while to test the acidity of two of the varieties of potatoes used in these experiments—Bliss Triumph, which is very susceptible to the disease, and McCormick, the variety which had proved rather resistant. Determinations of hydrogen-ion concentration were made on the expressed juice of tubers of these two varieties by the potentiometric method, and it was found that juice from the McCormick potatoes had a C_H 8.67×10^{-6} while that from Bliss Triumph had a C_H 8.63×10^{-7}. The McCormick had a hydrogen-ion concentration of about 10 times that of Bliss Triumph. To obtain further evidence on this point the fungus was grown in a series of potato-juice cultures made up to known hydrogen-ion concentration with $N^1/100$ sodium phosphate buffer mixture. The results of these experiments are shown in Table IV.

TABLE IV.—*Growth of Pythium debaryanum in potato juice of various hydrogen-ion concentrations*

C_H of culture medium.	Behavior of fungus.
3.936×10^{-9}	No growth in 3 days.
5.035×10^{-8}	1 grew in 3 days.
1.738×10^{-7}	2 grew well in 3 days.
1.660×10^{-6}	Growth covered plates in 3 days.
9.84×10^{-6}	Do.
2.535×10^{-5}	Do.
5.585×10^{-5}	Do.
3.741×10^{-4}	Do.
8.69×10^{-4}	5 mm. growth in 3 days.

According to Table IV the fungus grows well and fruits in a C_H 3.741×10^{-4}, which is considerably higher than that of the McCormick potato. The resistance of the McCormick potato to this disease, then, is not due to its high acidity. This is in accordance with the conclusions of Jones, Giddings, and Lutman (*18*) in regard to resistance of potatoes to *Phytophthora infestans*.

The experiments described in the foregoing pages seemed to indicate that the resistance to the progress of the fungus in McCormick potatoes might be due to some property of the cell wall—that is, it is possible that the fungus makes the opening in the cell wall through which it passes mechanically. If this is true, cell walls of potatoes resistant to the disease should show a higher resistance to puncture by mechanical means than the cell walls of susceptible varieties. This hypothesis seemed worth testing out, so an apparatus for measuring the pressure necessary to puncture the tissue of a potato was arranged.

This apparatus (Pl. 35)[3] consisted of a modified Joly balance, accurately graduated, and with a vernier for close reading. The lower end of the spring was attached to a metal rod which passed through a short glass tube fixed to the stand of the instrument. Hair lines on both the tube and rod made it possible to determine accurately the point at which the tension on the spring balanced any given weight. Tension was applied to the spring by means of a rack and pinion adjustment. It was possible with this balance to weigh to a milligram, which was well within the limits of experimental error in these determinations. A glass rod, the weight of which was less than the capacity of the balance, was suspended from the pan of the balance, and a small glass needle with a rounded end was attached to the lower end of the glass rod. In operating this apparatus a slice of potato was placed on the stage of the instrument, which was so adjusted that the tip of the needle just touched the surface of the potato when the hair line of the indicator on the spring coincided with the hair line of the fixed indicator of the balance. The tension on the spring was then slowly released by means of the rack and pinion adjustment until a sudden drop of the needle indicated that the tip of the needle had penetrated the tissue. The position of the column that supported the spring was then noted on the graduated scale. The weight required to balance the pull of the spring at this point was determined and subtracted from the weight of the needle. The result was the weight required to push the needle into the potato tissue.

Inasmuch as the needles used in these experiments were always from one-sixth to one-fourth the average diameter of potato cells, it is evident that in most cases at least the needle was pushed through the cell wall and that the weights obtained were a close approximation of the pressure necessary to break through the cell wall. The needles were drawn from small glass tubing over a micro burner and were drawn out in such a way as to leave a relatively heavy shoulder so that the slender portion which was thrust into the potato was not more than a millimeter in length. It was found that long needles of the small size necessary in this work were very easily broken. They were rounded and slightly larger at the end so that friction against the sides of the puncture would be reduced to a minimum. The needles used in these experiments were from 58.3 to 71 microns in diameter. In practice 20 determinations were made on each tuber, 10 in the cortex and 10 in the central portion within the ring of bundles. The weights obtained were averaged for each region; and, since the diameter of the needle was known, the weight required to break through tissue per square millimeter of surface was readily calculated. It was shown in this work by using different needles on the same potato that the weights required to puncture potato tissue were about propor-

[3] The authors are indebted to Mr. H. K. Sloat, of the Division of Illustrations, for photographing the motion pictures, and to Mr. J. F. Brewer, of the Laboratory of Plant Pathology, for preparation of the plates in this article.

tional to the area of the cross section of the needles. The determinations made with different needles are thus comparable.

In the experiments, the results of which are shown in Tables V to VII, inclusive, the potatoes were inoculated first in the cortex. This was done by removing a piece of the potato skin about 1 mm. in thickness and 5 to 10 mm. in diameter. A Van Tieghm cell was cemented to the surface of the potato around this wound with vaseline, and a drop or two of sterilized distilled water was placed within the ring and inoculated with mycelium of the fungus. The top of the cell was then closed with a cover glass and vaseline. The inoculated tubers were then placed in an incubator maintained at 30° C. and examined daily. If the tubers became infected, they were removed before they were more than half rotted. They were sliced through the point of inoculation, the distance the rot had progressed was measured, and the weight necessary to puncture the tissue in the two different regions of the sound portions of the tubers was determined. If the inoculation was unsuccessful, a second inoculation was made in the cortex in the same way as the first; and if this inoculation did not result in an infection the potato was inoculated beneath the cortex in the central part. If the results from this third inoculation were negative, as they usually were with McCormick potatoes, the tuber was considered to be immune, and the weight necessary to puncture the tissue was determined as described above. It is worthy of note that Bliss Triumph and Green Mountain potatoes usually rotted as a result of the first inoculation.

The tables in which the results of these experiments are given show the diameter of the needle in microns and the pressure required in grams per square centimeter to penetrate the tissue of the freshly cut potato in the cortex and central portion, respectively. In every case the numbers given as the pressure required to penetrate the tissue are averages of 10 determinations. The same needle was always used for both the cortex and the central portion. The number of inoculations made on each tuber, the region in which they were made, and the result after the length of time indicated are also given. In Table VII, under "results of inoculation," the term "slight rot" appears. This was used to characterize the results of inoculations when there was a browning and slight softening of the tissue immediately around the point of inoculation which seemed to indicate that infection had occurred. The rot had not progressed a measureable distance, however, and the tuber thus affected was apparently practically immune.

TABLE V.—*Pressure in grams per square centimeter required to puncture tissue of freshly cut surface of Green Mountain potatoes and results of inoculating these potatoes with Pythium debaryanum*

Tuber No.	Diameter of needle (in microns).	Pressure required to puncture cell wall.		Number of inoculations.	Location of final inoculation.	Results of inoculations.
		Cortex.	Central part.			
27	71	51,556.4	31,938.2	1	Cortex..	40 mm. rot in 3 days.
28	71	48,248.3	35,648.8	1	...do.....	30 mm. rot in 3 days.
29	71	37,747.1	32,072.7	1	...do.....	27 mm. rot in 3 days.
30	71	39,390.7	28,500.0	1	...do.....	40 mm. rot in 3 days.
31	71	57,571.5	47,774.0	1	...do.....	16 mm. rot in 3 days.
65	67	42,812.7	33,344.0	1	...do.....	30 mm. rot in 4 days.
66	67	47,566.3	42,667.0	1	...do.....	19 mm. rot in 3 days.
67	67	31,132.0	30,160.4	1	...do.....	34 mm. rot in 4 days.
68	67	34,439.6	34,749.0	1	...do.....	29 mm. rot in 4 days.
69	67	33,540.3	33,902.0	1	...do.....	49 mm. rot in 4 days.
70	67	41,344.0	25,736.6	1	...do.....	25 mm. rot in 4 days.
72	67	32,372.4	29,457.6	1	...do.....	25 mm. rot in 5 days.
73	67	42,965.1	39,525.0	1	...do.....	30 mm. rot in 5 days.
74	67	39,008.1	26,367.1	1	...do.....	33 mm. rot in 5 days.
75	67	31,276.4	22,843.6	1	...do.....	34 mm. rot in 5 days.
80	67	60,103.9	41,953.8	3	Deep....	12 mm. rot in 10 days.
61	67	75,525.1	28,351.7	3	...do.....	18 mm. rot in 4 days.
55	68.3	47,897.0	39,152.8	3	...do.....	18 mm. rot in 2 days.
Average		48,682.0	31,325.0			
Average for tubers which when inoculated in cortex rotted		40,731.3				
Average for tubers which when inoculated in cortex did not rot		61,175.3				

TABLE VI.—*Pressure in grams per square centimeter required to puncture tissue of freshly cut surface of Bliss Triumph potatoes and results of inoculating these potatoes with Pythium debaryanum*

Tuber No.	Diameter of needle (in microns).	Pressure required to puncture cell wall.		Number of inoculations.	Location of final inoculation.	Results of inoculations.
		Cortex.	Central part.			
32	71	40,548.1	30,345.4	1	Cortex..	32 mm. rot in 3 days.
33	71	51,457.7	27,923.0	1	...do.....	31 mm. rot in 3 days.
34	71	42,662.5	27,824.6	1	...do.....	Do.
35	71	39,380.2	30,656.5	1	...do.....	24 mm. rot in 3 days.
36	71	53,028.7	35,401.0	1	...do.....	19 mm. rot in 5 days.
39	71	45,953.9	34,946.0	1	...do.....	68 mm. rot in 5 days.
40	71	51,726.4	34,264.0	1	...do.....	42 mm. rot in 5 days.
41	71	40,332.1	36,610.2	2	...do.....	28 mm. rot in 4 days.
42	71	40,579.1	29,034.0	2	...do.....	46 mm. rot in 4 days.
43	71	37,798.6	29,892.0	2	...do.....	32 mm. rot in 4 days.
62	67	41,447.3	29,365.0	1	...do.....	18 mm. rot in 4 days.
63	67	34,501.5	22,504.4	1	...do.....	23 mm. rot in 4 days.
64	67	33,282.0	29,819.4	1	...do.....	24 mm. rot in 4 days.
71	67	33,282.0	22,677.2	1	...do.....	28 mm. rot in 4 days.
56	68.3	45,705.8	41,529.0	3	Deep....	5 mm. rot in 2 days.
57	68.3	40,000.3	30,343.4	3	...do.....	Do.
Average		41,980.0	38,209.0			
Average for tubers which when inoculated in cortex rotted		38,998.0				
Average for tubers which when inoculated in cortex did not rot		42,852.9				

TABLE VII.—*Pressure in grams per square centimeter required to puncture the tissue of freshly cut surface of McCormick potatoes and results of inoculating these potatoes with Pythium debaryanum*

Tuber No.	Diameter of needle (in microns).	Pressure required to puncture tissue.		Number of inoculations.	Location of final inoculation.	Results of inoculations.
		Cortex.	Central part.			
41	71	75,057.3	61,375.2	3	Deep....	Slight rot in 10 days.
46	71	86,357.1	62,615.5	3	...do....	Slight rot in 5 days.
47	71	105,644.3	82,429.2	3	...do....	Do.
48	58.3	112,803.3	67,111.7	3	...do....	Slight rot in 7 days.
49	58.3	77,189.2	55,835.1	3	...do....	Slight rot in 5 days.
50	58.3	100,362.2	62,284.9	3	...do....	No rot in 7 days.
51	58.3	138,760.5	113,923.0	3	...do....	Do.
53	58.3	67,566.4	51,101.6	3	...do....	10 mm. rot in 7 days.
54	58.3	78,119.5	54,243.3	3	...do....	12 mm. rot in 10 days.
83	67	51,824.7	48,083.2	1	Cortex.	35 mm. rot in 8 days.
Average		89,368.4	65,900.2			
Average for tubers which when inoculated in cortex did not rot		93,539.9				
Average for tubers which when inoculated in central part did not rot			72,224.9			
Average for tubers which when inoculated in central part rotted			52,672.4			

Tables V to VII show that there is considerable difference in the pressure required to puncture the tissue of the different regions of tubers of the three varieties used. If the pressures required to puncture the tissues of similar regions in the different varieties are compared, it is evident that the pressure is considerably higher for McCormick than for the two susceptible varieties, Bliss Triumph and Green Mountain, while the averages for the last two mentioned are in much closer agreement.

In regard to susceptibility to infection by the fungus, only 1 McCormick tuber out of 10 became infected when inoculated in the cortex, and the pressure required for puncturing the cortex of this tuber was much below the average required for the central portion of this variety. Two other McCormick potatoes became infected when inoculated in the central part, and these tubers were also lower in their resistance to puncture in this region than the others.

Three tubers of Green Mountain potatoes did not become infected even when inoculated twice in the cortex. The average pressure required for the cortex of these three tubers is 61,175.3 gm. per square centimeter, or considerably more than the average, 40,731.3 gm., required for the cortex of the tubers which rotted when inoculated in that region. All the tubers of the Green Mountain variety rotted. All the Bliss Triumph tubers, except two, became infected from cortical inoculations. These two required a somewhat higher pressure to puncture the tissue of the cortex than the average for this region, but the difference was not great. There is evidently a correlation between the resistance of the tuber to puncture and resistance to infection by the fungus.

The difference in resistance to puncture by mechanical means in these three varieties of potatoes is very marked; and it was considered of interest to see if there was correlated with it some variation in the chemical composition of the tubers, especially in the constituents of the cell wall. Accordingly, determinations of the pentosan and crude fiber content of the two regions, cortex and central portion, of potatoes of each of the three varieties were made.

In preparing the samples for analysis the potato was cut into slices about 8 mm. in thickness, a thin peeling removed with a sharp knife, and the cortex sliced away. This was dried, ground, and analyzed. The central portion of the potato after the ring of bundles had been peeled off was treated in like manner. Pentosan and crude-fiber analyses were made and were calculated to dry weight, the dry weights being obtained in the usual way by drying to constant weight in a vacuum oven. Duplicate determinations were made. The data obtained from the analyses are given in Tables VIII and IX.

TABLE VIII.—*Pentosan content of cortex and interior tissue of potatoes*

[Expressed as percentage of dry weight]

Location of tissue.	McCormick.	Green Mountain.	Bliss Triumph.
Cortex	2.00	1.60	1.86
	2.23	1.70	1.74
Interior	1.40	1.60	1.71
	1.32	1.70	1.65

TABLE IX.—*Crude-fiber content of cortex and interior tissue of McCormick, Bliss Triumph, and Green Mountain potatoes*

[Expressed as percentage of dry weight]

Location of tissue.	McCormick.	Green Mountain.	Bliss Triumph.
Cortex	3.42	2.01	1.98
	3.12	1.93	1.95
Interior	2.12	1.96	1.88
	2.18	1.83	1.92

From Table VIII it is evident that the pentosan content of the cortex of McCormick potatoes is somewhat higher than that for the other two varieties. The pentosan content of the central portion, however, seems to be somewhat lower in this variety. In Table IX is shown the crude-fiber content of the three varieties. McCormicks are higher in crude-fiber content than either of the other varieties. In the cortex of the McCormicks there is over 50 per cent more crude fiber than in the same region of the other two varieties. The interior also of the McCormick tubers

is higher in crude-fiber content than the cortex of Green Mountain or of Bliss Triumph. There is evidently correlated with resistance to mechanical puncture and resistance to infection by *Pythium debaryanum* a higher crude-fiber content.

Further evidence that the resistance of potatoes to infection was correlated with resistance of the tissue to mechanical puncture was obtained from experiments in which Bliss Triumph and Green Mountain tubers were prepared for inoculation by scooping out small plugs of tissue from the cortex of the tubers and allowing the wounds to dry for a given length of time. The plugs were removed by means of a small curved knife, leaving a cavity in the cortex of the tuber about 4 mm. in diameter and of the same depth, without sharp corners or rough surfaces. Part of these potatoes were inoculated as controls, and all of them were placed in the incubator at 30° C. At 3-hour intervals for 12 hours a number of the uninoculated tubers were removed from the incubator, inoculated in the cavities made at the beginning of the experiment, and replaced in the incubator. The inoculations were made in the usual way by placing a bit of mycelium in a drop or two of sterilized water in the cavity, which was then inclosed in a covered Van Tieghm cell. When at the end of 4 days the tubers were removed from the incubator and examined, it was found that only the controls inoculated when the experiment was set up had rotted. None of the wounded potatoes inoculated 3 hours or more after they had been placed in the incubator were rotting. Apparently exposure to the air at 30° C. for 3 hours was sufficient time for the formation of a layer over the wound resistant to fungus attack.

It is commonly considered by the potato growers of the San Joaquin Valley, Calif., that wounds which have had opportunity to cork over will not become infected. This has been shown to be true in these studies by many unsuccessful attempts to inoculate tubers in old wounds. From the experiments described in this paper it is evident that the protective covering is formed very quickly under the conditions of the experiment. Appel(1) claims that the tissue of some varieties of potatoes begins to cork over in 6 hours. That a protective covering is formed in 3 hours under the conditions of the experiment is evident. There is, however, no evidence that it is a true suberization.

The pressure necessary to puncture the tissue of these Green Mountain and Bliss Triumph potatoes was determined on freshly cut slices of the tubers and on slices which had remained in the incubator for 3 hours. The results are shown in Table X.

TABLE X.—*Pressure in grams per square centimeter required to puncture tissue in slices of potatoes freshly cut and after drying for 3 hours at 30° C.*

GREEN MOUNTAIN

Potato No.	Cortex.			Interior.		
	Fresh surface.	Dried 3 hours.	Average increase in pressure required for puncturing dried tissue.	Fresh surface.	Dried 3 hours.	Average increase in pressure required for puncturing dried tissue.
1	40,852.1	59,225.1	.....	30,554.8	58,388	.....
2	47,738.9	72,409.8	.....	30,554.3	52,737.8	.....
3	39,553.2	68,851.9	.....	22,528.2	57,551	.....
4	43,111	76,385.9	.....	31,810	70,344.8	.....
5	27,833.7	74,711.7	.....	32,019.2	48,552	.....
Average	39,017.7	70,316.8	31,299.1	29,493.3	57,514.7	28,021.4

BLISS TRIUMPH

1	42,528.3	76,176.8	.....	29,994.8	48,133.6	.....
2	47,715	79,315.9	.....	27,624.4	46,250.1	.....
3	41,018.1	64,456	.....	22,601.9	78,068	.....
4	38,448.3	57,132.6	.....	33,065.6	49,644.2	.....
5	35,577	60,690.1	.....	26,368.8	57,715	.....
Average	41,057.3	67,554.2	26,496.9	27,931.1	55,962.1	28,031.0

From the results shown in Table X it is evident that the resistance of the wounded surface of the potato to puncture is appreciably increased in every instance by exposure to the air for 3 hours. In the five Green Mountain potatoes the average increase in pressure required to puncture the cortical tissue was 79 per cent, and the average increase for the central tissue was 95 per cent. With the same number of Bliss Triumph tubers the results were 64 per cent and 100 per cent for the cortex and central portions, respectively.

There is then correlated with the resistance to infection shown by wounds after 3 hours' drying a very marked resistance to mechanical puncture. If the fungus penetrates the tissue mechanically, it is quite possible this increase in resistance, due to drying, would be sufficient to prevent its entrance. It is noticeable that the pressure required to puncture the dried cortex, the region in which the inoculations were made in these experiments, most closely approaches the averages for the inner portion of McCormick tubers which did not become infected.

Another point which is of interest in this connection is the fact that no cases of natural infection through the potato skin have been observed, and repeated attempts in the laboratory to inoculate tubers on the surface have yielded negative results. Correlated with this resistance to infection is a very marked resistance to mechanical puncture. It was exceedingly difficult to puncture the skin of the potato with the round-

tipped glass needles, and the pressure required was considerably more than that required for any portion of the cut surface of tubers tested.

No direct evidence was obtained that the fungus could exert sufficient pressure upon the cell walls of susceptible potato tubers to puncture them. However, some indirect measurements were made of the pressure the fungus might be capable of exerting under certain conditions. When fungus filaments were plasmolyzed in cane sugar solution it was found that it required a solution capable of exerting about 54 atmospheres, or 55,773.3 gm., per square centimeter to plasmolyze them. If, then, the protoplasm of the fungus is not permeable to cane sugar, the filaments are capable of withstanding nearly 55,773.3 gm. pressure per square centimeter; or, stated in another way, the filaments are capable of exerting that much pressure. This is considerably more pressure than is required to puncture the tissue of the central parts of Bliss Triumph and Green Mountain potatoes. It is sufficient pressure to puncture the cell walls of the cortex of all tubers of these varieties which rotted when inoculated in that region except one. This exception is tuber 31 in Table V, a Green Mountain tuber which required 57,571.5 gm. per square centimeter, or 1,798.2 gm. more than the osmotic pressure of the fungus filament as found in this study. It is also sufficient to puncture the tissue of the two McCormick tubers which rotted when inoculated in the central portion and the one which rotted when inoculated in the cortex.

The pressure would not be sufficient to puncture the cell walls of McCormick tubers when they were resistant to infection, and it is lower than that required for the cortex of two of the three Green Mountain tubers that did not rot when inoculated in that region. The third Green Mountain and the two Bliss Triumph tubers that did not rot when inoculated in the cortex required pressures considerably below the osmotic pressure of the fungus filament to puncture the cell walls. Just why these three potatoes did not rot is not apparent. It is, of course, possible that the 10 determinations of the pressure required to puncture cells of the cortex were made on less resistant cells than those upon which the inoculations were made. Another possibility is that a weak culture of the fungus was used. These 3 potatoes, however, were exceptions to the rule.

The experiments for the determination of the pressure required to puncture the tissue of the potatoes on the fresh surface and when dried at 30° C., as detailed in Table X, show that the pressures required for the cortex of the dried tubers, which were resistant to infection, were considerably higher than the osmotic pressure of the fungus filament. This is in agreement with the evidence just brought out from data in Tables V to VII. It would seem from this work that the mechanical pressure of the fungus filament against the cell wall of the potato is an important factor in the penetration of the potato tissue by the fungus.

One consideration detracts from the value of this indirect method for the determination of the pressure the fungus filament is able to exert against the cell walls. In the osmotic pressure determinations, an attempt was made to determine the total pressures within the filaments of the fungus, and these may or may not be the pressure the fungus is able to exert against the cell wall of its host plant. The cell walls of the fungus filament are apparently able under ordinary conditions to withstand the pressure within the filament, except at the growing points. The pressure exerted on the cell wall of the potato under the most ideal conditions would be the pressure that the contents of the filaments

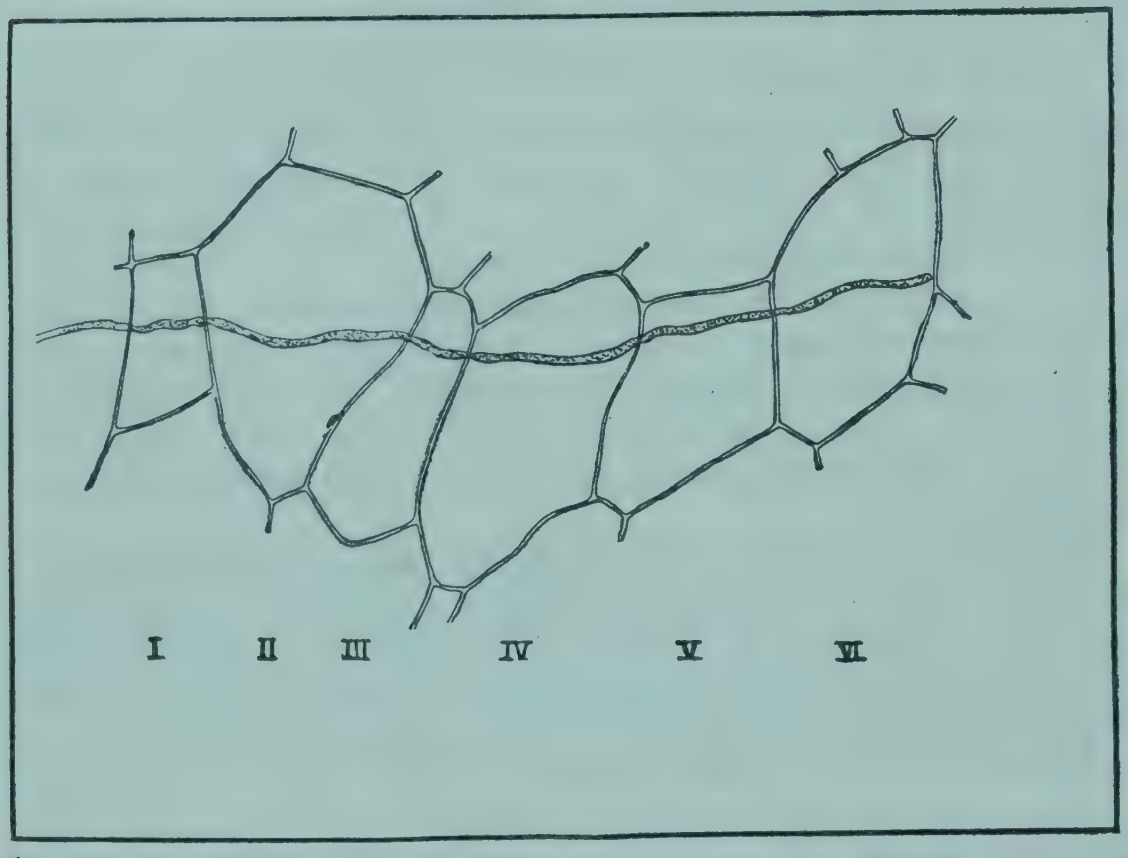

FIG. 1.—Drawing to illustrate growth of a Pythium hypha in potato tissue. Note the constriction of the hypha where it penetrates the wall.

were capable of exerting minus the pressure necessary to push out the wall or rudimentary wall of the tip of the fungus filament.

Further evidence on the method by which the fungus penetrated the cells of the potato was furnished by direct observations of the hyphae of the fungus within the tissue of the potato. In these experiments sections of raw potato were prepared as nearly sterile as possible and inoculated with the fungus. When kept overnight in hanging drop cells at 30° C. a good growth of hyphae was usually obtained. Numerous instances of cell-wall penetration were observed, and the method of penetration was followed both by serial drawings and by motion photomicrographs (Pl. 36, 37). The part of the section selected for observa-

tion was usually two or three cells thick, since if the section is much thinner the hyphae are liable to grow over the surface.

The hypha shown in figure 1 was watched continuously for three hours. During this time it grew 1,976 μ at a room temperature of about 70° F. The time required to penetrate the wall was about 5 minutes. The distance traversed and the time required for each cell were as follows:

The hypha in cell I, traversed 200.3 microns in 25 minutes.
The hypha in cell II, traversed 493.9 microns in 35 minutes.
The hypha in cell III, traversed 186.9 microns in 20 minutes.
The hypha in cell IV, traversed 387.2 microns in 20 minutes.
The hypha in cell V, traversed 333.7 microns in 20 minutes.
The hypha in cell VI, traversed 373.8 microns in 30 minutes.

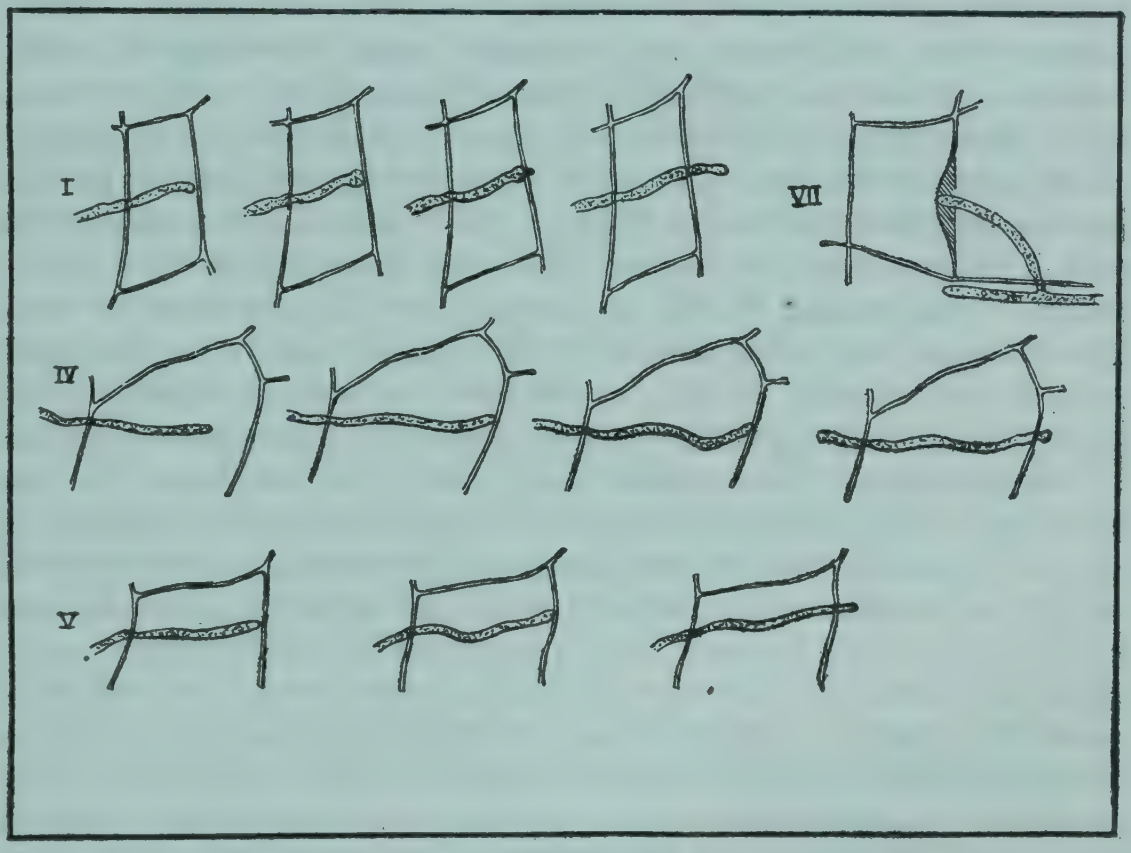

FIG. 2.—Drawing to illustrate method of cell-wall penetration in cells I, IV, and V. For explanation see text.

The drawings of figure 2, made at the time of observation to show the relative positions assumed, give the characteristic methods of cell-wall penetration as observed in this study. In passing through the cell wall between cells I and II the hypha approached the cell wall nearly at right angles; it formed a swelling at the end, bent slightly, and penetrated the wall by a small tube. After passing through the cell wall into the next cell the hypha expanded to its usual diameter. Considerably more bending of the hypha is shown in cells IV and V. It is noticeable that the wall in cell V bends outward under the pressure of the hypha and that the hypha straightens after the wall is penetrated.

There is quite clear evidence of the exertion of mechanical pressure. In cell VII another hypha was observed growing toward the potato cell wall. At first the hypha was straight; then as growth pressed it against the wall it bent upward, at the same time making a dent in the wall near the center of the wall face. This hypha did not penetrate the wall, for when it had reached the position given in the figure, rapid growth was begun by the growing tip just below it, and the pressure of the upper hypha seemed to be insufficient to break the wall.

Where the hypha approaches the cell wall at right angles it usually passes through as shown in cell I; but when the tip strikes the wall obliquely it does not usually penetrate but pushes along the wall and may go entirely around the cell, forming a coil within it. In traveling around the cell the tip may reach a corner, in which case the wall is frequently penetrated and the hypha grows between the cells. This is characteristic, and hyphae are frequently found following the middle lamellae. When the growth of the young tip is stopped for a few moments as by strong light, a cell wall is apparently formed over the tip; and on the resumption of growth this wall is broken at its weakest point by the pressure developed within the hypha. The formation of a strong hyphal wall requires about two minutes under the conditions of these experiments. The swelling of the tip shown in cell I took place in about two minutes—that is, the cell wall of the hypha seemed to become strong enough in that length of time to withstand the pressure within. There must, then, be a rapid transformation of the fluid protoplasmic material to form this wall. This transformation may be of the nature of a precipitation at the boundary between the hyphal sap and the potato cell sap. It is possible that the precipitation of substances to form the strong hyphal wall occurs only in contact with the cell sap of the potato, at least occurs more rapidly in contact with the potato cell sap than in contact with the cell wall. If this is true the hypha would form a tube of plastic materials against the cell wall and the growth pressure of the fungus filament would be applied directly to the cell wall of the potato. This might be the explanation of the mechanics of cell-wall penetration by the fungus. Evidence that the hyphae are sometimes cemented fast to the cell wall was secured during observations of hyphae that strike the cell wall obliquely, slide along it for a short distance, and then stop and penetrate the wall.

DISCUSSION OF RESULTS

In a consideration of the results brought out in the foregoing pages it is apparent that there is much evidence that the fungus makes its way through the cell walls of the potato mechanically. It is, of course, impossible to prove in work of this kind that some enzym is not secreted at the tip of the hypha which softens or destroys the portion of the cell wall with which it is directly in contact. If, then, no evidence of the

existence of such an enzym was brought out in this study, the fact that the fungus requires such a short time (about five minutes) to pass completely through a cell wall seems to indicate that the main factor, at least in the breaking through the cell wall, is mechanical pressure, for in enzym action it would be necessary to have a diffusion of the enzym from the tip of the hypha into the cell wall and at least a softening, if not a dissolving, of a portion of the cell wall at that point. Whether a substance, such as an enzym, with a relatively high molecular weight and consequently low rate of diffusion could diffuse into and through the cell wall with sufficient rapidity to soften or dissolve this tissue in the time required for the fungus to pass through the cell wall is doubtful. Another point in support of the hypothesis that the opening in the cell wall is made mechanically is that there is apparently no considerable increase in the size of the opening after the tip of the hypha passes through. If the fungus secretes an enzym which acts on the cell wall, it would seem probable that this enzym action would continue after the tip of the hypha passed through and the opening would be larger than the hypha. Hasselbring (*13*) has figured the breaking down of the host tissue around the fungus hypha. This phenomenon is common where a fungus breaks down the cell walls of its host enzymically. With *Pythium debaryanum* on potato, however, the opening in the cell wall is never larger than the mean diameter of the hypha in the lumen of the cells and is usually considerably smaller.

Another point which supports the hypothesis of the mechanical puncture of the cell walls of the host by the fungus is the fact that apparently only the middle lamella of the potato cell wall is affected. The fungus seems not to break down the secondary thickening of the cell walls, and when a piece of well-rotted potato is teased out on a slide the cells full of starch grains float free.

If, then, as seems probable, the fungus makes its way through the cell walls by mechanically puncturing them, a potato with cell walls strong enough to withstand the pressure exerted by the fungus would be immune to the disease. If we consider the osmotic pressure within the fungus filament—as determined in this study—as the pressure the fungus is able to exert against the wall of its host plant, then the resistance of potatoes in all cases in which they did not become infected would be explained, with the three exceptions mentioned earlier. This would also account for the infection of all potatoes which became infected in either the central portion of the tuber or the cortex, with the exception of one Green Mountain tuber. Another point which should be noted in this connection is that correlated with this resistance is a higher crude-fiber content in the McCormick. This is probably due to more secondary thickening in the cell walls. It is quite possible that the White McCormick potato or some hybrid of this variety would be resistant to the fungus when grown in the San Joaquin Valley and would thus solve

the problem of the control of this disease, but no field tests have been made for varietal susceptibility.

If the fungus enters the potato cell by breaking through the cell walls mechanically, it is of course necessary that there be some support from which this pressure may be developed. This support would be readily furnished where the fungus filaments were against the opposite cell wall—for instance, when the fungus is within the tissue. It seems probable also, as has been brought out earlier in this paper, that the fungus may attach itself to the cell walls of its host. In the penetration of the host tissue from the outside, as it took place in this study, it is of course necessary to have an attachment of the fungus hyphae to the host tissue. This may be accomplished by the newly formed wall of the fungus hyphae adhering to the cell wall of the potato. Blackman and Welsford (*5*) considered that the mucilaginous membrane of the germ tube of Botrytis formed an attachment sufficiently strong to withstand the pressure necessary for the puncturing of the cuticle of broad bean leaves. In natural infections the fungus hyphae are frequently thrust deep into the tissues of the potato, and a support from which the pressure could be developed would readily be found by the closing of the wound in the tuber.

If in its growth in the potato this fungus breaks its way through the tissue mainly by mechanical means, as seems quite possible, it is in keeping with the manner in which roots grow through potato tissue. Peirce (*24*) has shown that roots of *Pisum* sp. and *Vicia faba* can force their way through potato tissue mechanically, and one of the present writers has frequently observed potatoes in the San Joaquin Valley with roots growing through them. A somewhat analagous condition is found in the penetration of the stigma and style of certain Rubiaceae by the pollen tube, as described by Lloyd (*19*).

While it has not been proved in this investigation that *Pythium debaryanum* penetrates the cell walls of the potato by mechanical pressure, there is considerable evidence that the main factor in this penetration is the growth pressure of the fungus filament and that the resistance of the White McCormick potatoes to this disease is due to cell walls that are more resistant to mechanical puncture than are the cell walls of extremely susceptible varieties.

SUMMARY

(1) It has been shown in this paper that *Pythium debaryanum* destroys the pentosans, starch, and sugar of the potato tuber in rotting it.

(2) The fungus secretes a toxin which kills the cells of the potato. It also secretes an enzym which breaks down the middle lamellae of the cells but apparently has little or no effect on the secondary thickening.

(3) More pressure was required to puncture the tissues of White McCormick potatoes, which are comparatively resistant to the disease,

than to puncture the tissues of the two susceptible varieties, Bliss Triumph and Green Mountain. Correlated with this resistance to puncture is a resistance to infection by *Pythium debaryanum*.

(4) The resistance to puncture in McCormick tubers is also correlated with a higher crude-fiber content, which was considered to be due to more secondary thickening in the cell walls.

(5) The cut surface of the cortex of Bliss Triumph and Green Mountain when dried for three hours was much more resistant to puncture than the freshly cut surface. Here also there is a correlation between resistance to infection by this fungus and resistance to mechanical puncture.

(6) The osmotic pressure within the fungus filament, as determined by plasmolysis in this work, was sufficient to develop the pressure necessary to puncture the cell walls in the potato tubers in all cases in which infection occurred, with one exception. It was not sufficient to develop the pressure necessary to puncture the tissue of the potatoes in the cases where no infection occurred, with three exceptions.

(7) Mechanical pressure exerted by the fungus hyphae seems to be the most important factor in cell-wall penetration by this fungus, and resistance to infection is apparently due to resistance of the cell walls to mechanical puncture. Microscopical observations of cell wall penetration by the fungus hyphae seem to corroborate this theory.

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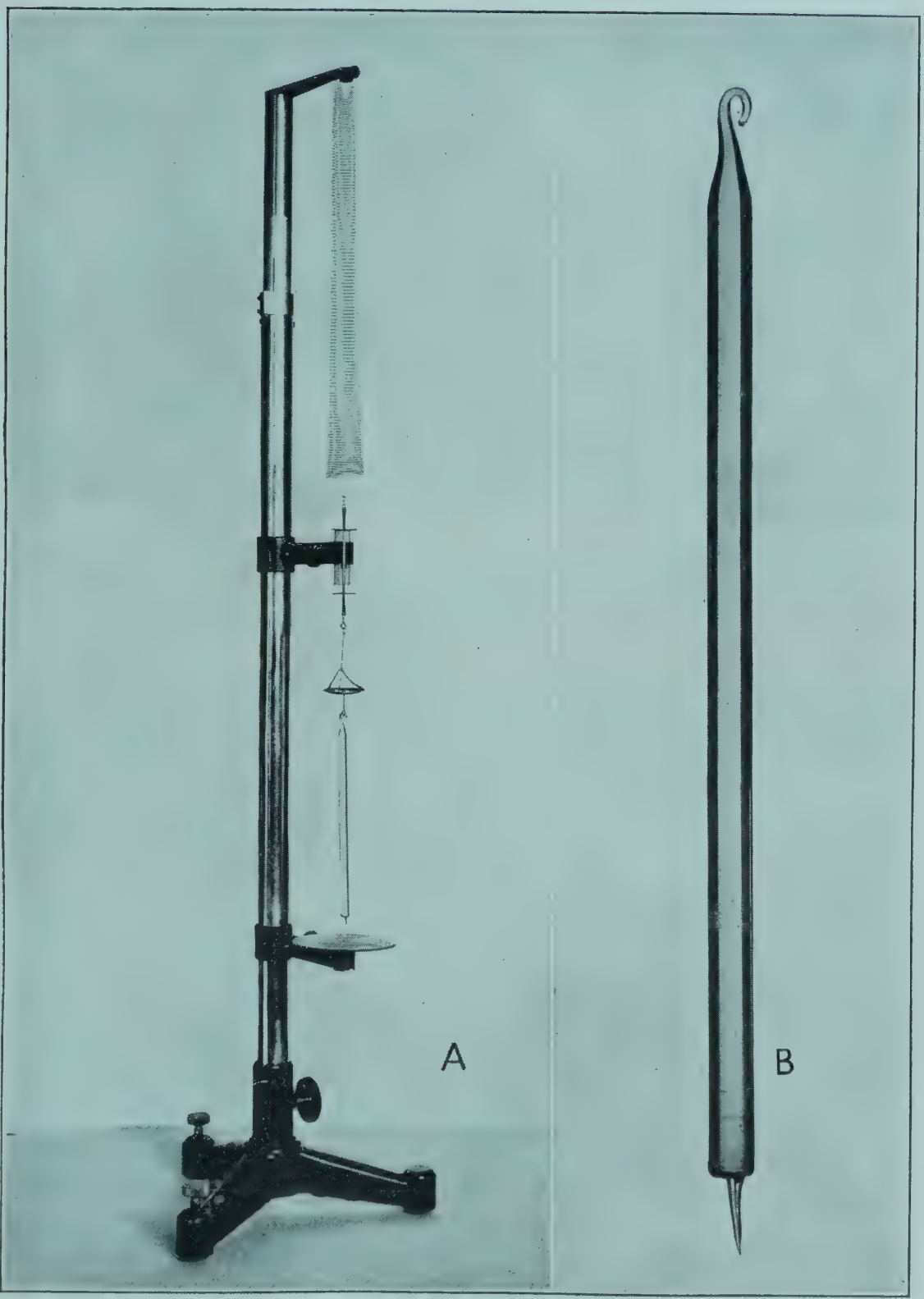
PLATE 35

A.—Apparatus used in determining the pressure required to puncture the tissue of potato tubers.

B.—Glass rod with attached needle. About actual size. Photographs by J. F. Brewer.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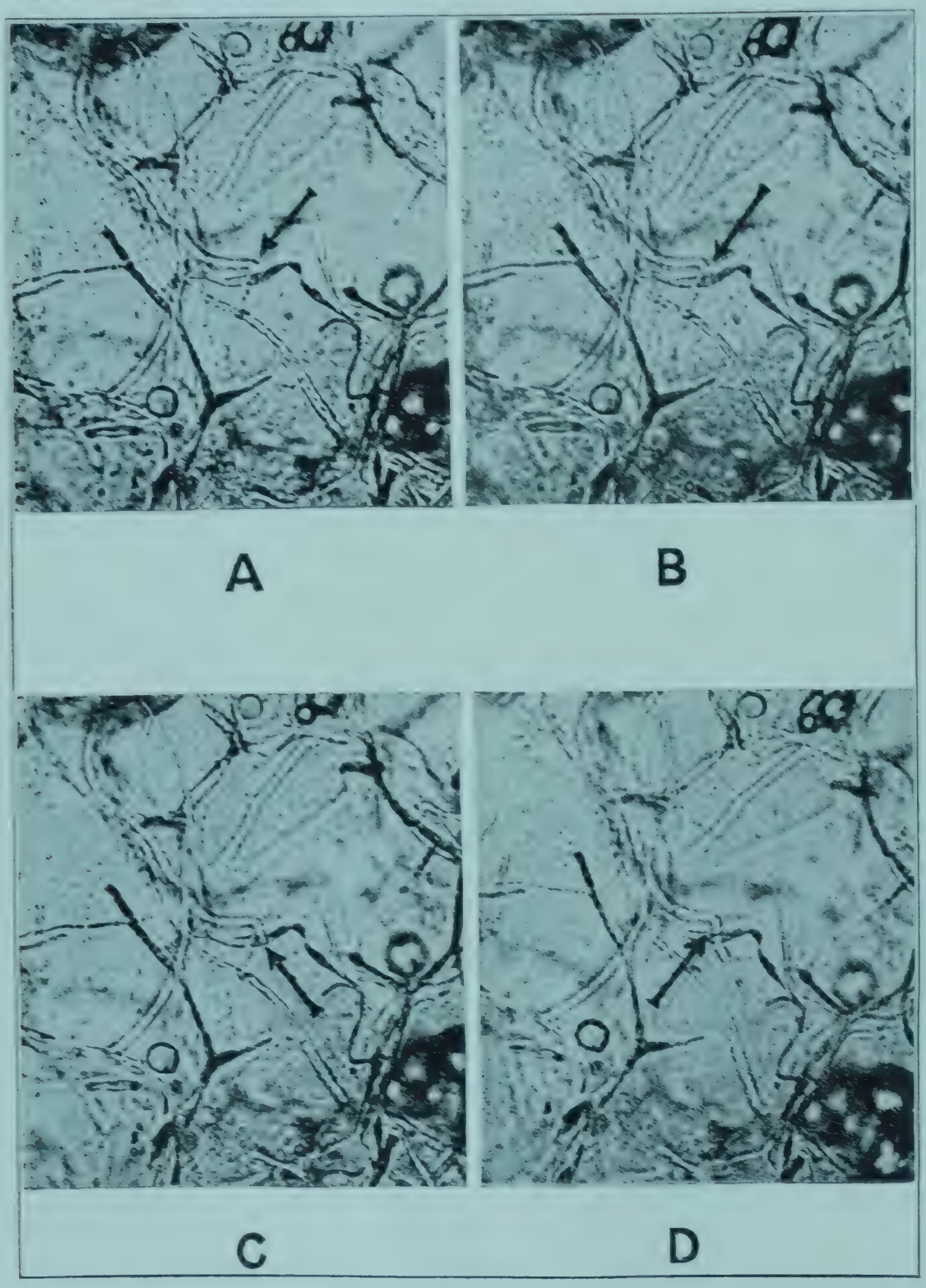
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PLATE 36

Photographs enlarged from portions of a motion photomicrograph, showing the method of cell wall penetration by Pythium hyphae.

A.—Shows hypha growing toward the potato cell wall.

B.—Shows hypha attached to wall and about to penetrate.

C.—The tip has just broken through the wall.

D.—The penetration is complete. Note the black line at the point where the hypha penetrates the wall. This may be due to a rolling up of the potato cell wall about the hypha or to a difference in refraction caused by compression of the wall.

PLATE 37

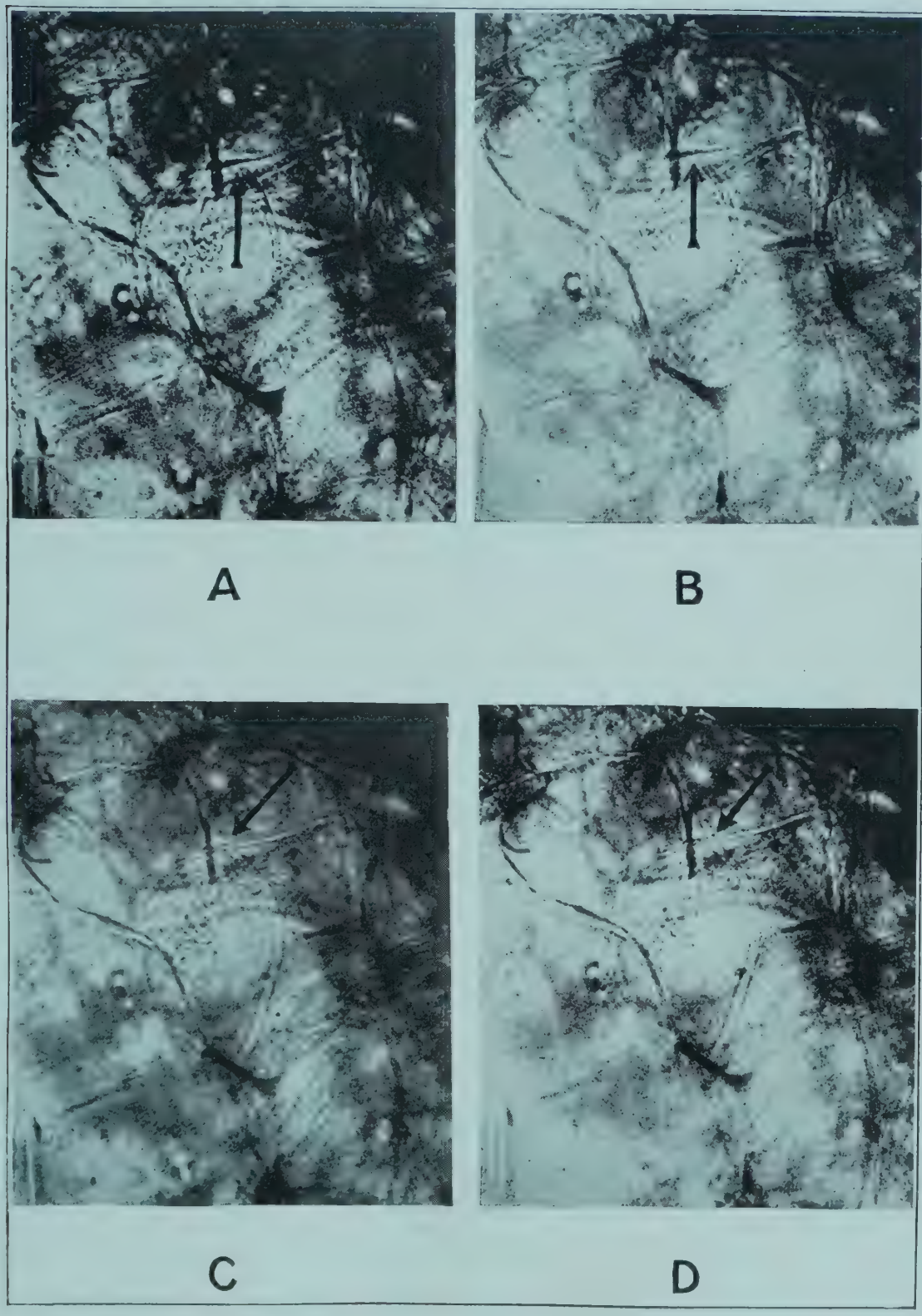
Photographs enlarged from portions of a motion photomicrograph, showing the method of cell wall penetration by Pythium hyphae.

A.—Shows the hypha growing against the potato cell wall. Sufficient pressure has already been applied to cause the hypha to bend. Notice that this bending increases in later photographs.

B.—A little later stage than A.

C.—The tip has broken through as a small tube.

D.—Penetration is complete. Notice the constriction of the hypha at the point where it penetrates the potato cell wall.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LOSSES OF ORGANIC MATTER IN MAKING BROWN AND BLACK ALFALFA[1]

By C. O. SWANSON, L. E. CALL, and S. C. SALMON, *Kansas Agricultural Experiment Station*

Large losses of alfalfa [2] due to improper curing of the first crop have led to the employment of methods other than that of curing in the field and stacking. Some farmers convert the green alfalfa into silage, but there are so many difficulties [3] in making good silage from alfalfa that this method is rarely practised. Others stack the alfalfa in a partially wilted condition. The great weight excludes the air, and fermentations occur somewhat similar to those which occur in a silo. The product is known as brown and black alfalfa. The degree of color depends upon the conditions which control the nature and extent of the fermentations. Some of these conditions are moisture content of the alfalfa when stacked, size and shape of the stack, and temperature and rainfall during the time of curing. Such alfalfa, according to growers who use this method, is relished by cattle; and some practical feeders consider it superior to ordinary alfalfa hay.

However, when fermentation occurs there is evidently a loss in nutritive value. Since the nature and amount of these losses apparently were unknown, the writers decided to investigate them and also to compare the feeding value of black and brown alfalfa with that cured in the usual way.

For the purpose of this experiment a uniform field of alfalfa, estimated to make a 45- to 50-ton stack, was selected. The alfalfa was cut, wilted for a few hours and stacked in the open, each load being weighed separately. Some wilting was considered necessary in order to get a desirable product and also because hay loaders will not work satisfactorily in unwilted alfalfa.

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[1] Contribution from the Department of Agronomy (paper No. 16) and the Department of Chemistry of the Agricultural Experiment Station of the Kansas State Agricultural College. The Department of Animal Husbandry conducted the feeding trials. The chemical work was done in the analytical laboratory in charge of Assistant Professor W. L. Latshaw.

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Samples of 10 to 20 pounds each were taken from the different loads as the alfalfa was hauled to the stack. These were placed in bags and sent at once to the chemical laboratory. Here they were weighed again and the contents removed from the sack and spread out to dry. Care was taken to prevent any loss. When the samples were air-dry they were weighed again and passed through a feed cutter, and the moisture in the air-dry material was determined. The total moisture in the original samples was then calculated and was found to vary from 29 to 70 per cent, the average being 53.28 per cent. The percentage of feed constituents in the dry material was as follows: Ash, 9.27; protein, 17.25; crude fiber, 38.97; and ether extract, 2.68.

A few samples of the freshly cut alfalfa were also taken. The moisture content of these varied from 70 to 77 per cent and averaged 72.1 per cent. These variations illustrate some of the difficulties of conducting the experiment and should be considered in interpreting the results. The range in moisture content can be seen from the percentages given in Table I.

TABLE I.—*Percentage of moisture and dry matter in samples taken at time of stacking*

Sample No.	Description.	Total moisture.	Dry matter.
359	Alfalfa ready to load	69.36	30.64
360	.....do	71.05	28.95
362	.....do	66.94	33.06
365	.....do	52.43	47.57
366	.....do	53.21	46.79
368	.....do	57.55	42.45
373	.....do	29.25	70.75
374	.....do	38.68	61.32
381	.....do	50.52	49.48
382	.....do	41.06	58.94
386	.....do	56.04	43.96
361	Alfalfa just cut	77.22	22.78
364	.....do	71.55	28.45
363	.....do	75.22	24.78
367	.....do	71.20	28.80
385	.....do	69.49	30.51
	Average of all	59.42	40.58
	Average of "ready to load"	53.28	46.72

The alfalfa remained in the stack till early winter, when the stack was measured into four quarters. The plan was to leave one quarter intact until early spring. The alfalfa from the three other quarters was used in a feeding experiment with steers in which the black alfalfa from this stack was compared with good quality green alfalfa and also good quality brown alfalfa. Three samples were taken the latter part of December from the material and fed to steers. The last quarter was loaded and weighed the last part of March, and at which time the different kinds of

alfalfa present in this last quarter were sampled. It was assumed that the last quarter represented the whole stack, so the weights of the different kinds of hay removed from the stack were multiplied by four to obtain the weight of each kind of hay in the whole stack.

The total weight of hay removed from the stack, the estimated amounts of each kind, and the analysis of each kind, based on the samples taken the last of March from the last fourth of the stack, are given in Tables II and III.

TABLE II.—*Composition of samples of brown and black alfalfa taken from the stack*

Sample No.	Date of sampling.	Description of sample.	Total moisture.	Ash.	Protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
			Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
581	Dec. 28, 1916	Black alfalfa, charred, inferior quality.	70.39	3.99	5.55	11.91	7.66	0.50
582	.....do.....	.....do.....	61.84	6.35	7.37	11.21	12.37	.86
591	.....do.....	Black alfalfa, good quality.....	63.40	4.60	6.57	10.63	14.00	.79
592	Mar. 20, 1917	.....do.....	56.28	5.79	7.44	11.94	17.57	1.08
600	Mar. 28, 1917	Charred dry, not moldy.....	15.70	13.50	16.64	23.29	29.12	1.72
601	.....do.....	Partly moldy, but moist, second grade	48.54	13.40	10.53	12.83	13.81	.76
602	.....do.....	Black alfalfa, good quality.....	52.45	7.40	9.06	15.62	14.52	1.03
603	.....do.....	From stack bottom, bad odor.....	65.80	6.71	4.62	11.39	10.39	.99
604	.....do.....	Alfalfa hay, color and odor good.....	58.17	5.94	6.84	14.66	13.13	1.10
605	.....do.....	Dark brown hay, next to charred portion.....	5.73	13.62	17.53	21.47	40.14	1.56
606	.....do.....	Moldy, mostly charred.....	32.99	14.33	17.81	12.84	21.89	.87
607	.....do.....	Green hay from outside of stack, good.	5.02	10.39	13.65	31.99	37.95	.73
608	.....do.....	Light brown hay.....	3.80	14.20	16.39	28.20	36.56	.91

TABLE III.—*Weight of dry matter and chemical constituents of brown and black alfalfa taken from the stack, compared with the amounts put into the stack*

Sample No.	Description of sample.	Hay.	Dry matter.	Ash.	Protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
		Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
600	Charred hay.....	20,440	17,231	2,759	3,401	4,760	5,952	352
601	Partly moldy hay.....	8,040	4,137	1,077	846	1,031	1,110	61
602	Black hay.....	9,620	4,574	711	872	1,503	1,397	99
603	Stack bottom, bad odor.....	1,680	575	112	78	191	175	17
604	Green hay (?).....	6,000	2,510	356	410	880	788	66
605	Dark brown hay.....	5,440	5,128	741	954	1,168	2,184	85
606	Moldy and charred (?) on top of stack..	5,400	3,619	774	962	693	1,182	5
607	Green hay from the outside, good.....	4,120	3,913	428	562	1,317	1,564	30
608	Light brown hay.....	7,400	7,119	1,051	1,213	2,087	2,705	67
	Total taken from stack.....	68,140	48,806	8,009	9,298	13,630	17,057	782
	Original hay as put into the stack.....	171,480	80,115	7,426	13,819	25,500	31,220	2,147
	Loss.....	.....	31,309	[a] 583	4,521	11,870	14,163	1,356
	Percentage of loss.....	.....	39.07	[a] 7.84	32.71	46.54	45.36	63.57

[a] Gain. This gain of ash is not large considering the nature of the experiment and the assumption made in the calculations.

LOSSES OF ORGANIC MATTER

The total weight of the partially wilted alfalfa put into the stack was 171,480 pounds, of which 80,115 pounds were dry matter as determined by the average dry-matter content of all samples, which was 46.72 per cent.

The weights of the different kinds of material removed from the stack (Table II) totaled 68,140 pounds, of which 48,806 pounds were dry

matter. Comparing this with the 80,115 pounds of dry matter put into the stack, there was a loss of 31,309 pounds, or nearly 40 per cent.

The loss was calculated also on the basis of the chemical composition of the samples taken from the stack as compared with that of the alfalfa when it was put into the stack. It was assumed that there was no gain nor loss of ash. This assumption is probably more nearly correct for the inside than for the outside of the stack, where there may have been some loss by leaching and some addition from dust blowing into the stack. The two would to a certain extent balance each other. The following method of calculation was used: The average of all samples of the alfalfa as it went into stack shows that 100 pounds of alfalfa on a dry basis contained 9.27 pounds of ash. Alfalfa from the stack represented by sample 592 contained 13.24 pounds of ash in 100 pounds of dry matter. This increase in ash of nearly 4 per cent can be explained only by a loss of organic matter. This loss may be calculated by the proportion

$$9.27 : 13.24 :: 100 : x$$

in which $x = 142$. That is, 100 pounds of the alfalfa which was taken from the stack contained as much ash as 142 pounds when it was put into the stack. In other words, there was a loss of 42 of the 142 pounds, or practically 30 per cent for this particular sample. The loss for each kind of hay taken from the stack was calculated separately in a similar way. The average loss of total organic matter for all samples determined in this way was 39.2 per cent. The loss of protein, crude fiber, nitrogen-free extract, and ether extract was calculated by computing the amount of each of these constituents in 142 pounds of the original material and then comparing these amounts with the amounts present in the 100 pounds of material to which the original 142 pounds had been reduced. The data obtained in this way are given in Table IV.

TABLE IV.—*Losses of organic matter, calculated on the basis of the ash content of the alfalfa put into the stack and of the material taken from the stack*

Sample No.	Date of sampling.	Description of sample.	Total organic matter.	Protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
			Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
581	Dec. 28, 1916	Black alfalfa, charred, inferior	31.03	25.07	12.87	54.18	56.28
582	.....do.....	.....do.....	44.13	37.46	48.46	53.49	53.12
591	.....do.....	Black alfalfa charred, good quality.....	25.92	22.92	32.35	27.30	40.44
592	Mar. 20, 1917	.....do.....	30.07	31.03	39.99	27.88	35.77
600	.....do.....	Charred, dry, not moldy	41.86	33.46	49.52	48.43	55.53
601	.....do.....	Partly moldy, but moist, second grade...	64.44	57.76	72.11	75.50	80.34
602	.....do.....	Black alfalfa, good quality.....	40.47	34.26	38.58	53.34	51.99
603	.....do.....	From stack bottom, bad odor.....	52.60	62.85	50.37	63.04	48.65
604	.....do.....	Alfalfa hay, color and odor good.....	34.64	38.00	28.02	47.34	35.61
605	.....do.....	Dark brown hay, next to charred portion	35.90	30.88	54.14	29.96	60.52
606	.....do.....	Moldy, mostly charred	56.71	33.29	73.89	63.66	97.87
607	.....do.....	Green hay from outside of stack, good..	15.25	29.42	10.33	13.09	75.62
608	.....do.....	Light-brown hay.....	37.11	37.87	42.07	38.65	77.69
		Average.....	39.2	36.5	42.5	45.8	59.2

The results secured from the two methods of calculation agree remarkably well, considering that there are possibilities of error in sampling, that the figures given in Table IV depend on the assumption of no gain or loss of ash, and that the data presented in Table II are based on one-fourth of the stack only.

The first method of calculation (Table III) shows a loss of 39.1 per cent of material as compared with 39.2 per cent secured by the second method (Table IV). The corresponding figures for the percentage of loss of protein were 32.7 and 36.5, for the crude fiber 46.5 and 42.5, for the nitrogen-free extract 45.4 and 45.8, and for the ether extract 63.6 and 59.2. The largest loss appears to have been in the ether extract, and the smallest loss in the protein. The losses of all organic materials were large.

The description of the samples does not permit accurate comparisons of the losses that occurred in the different kinds of hay produced by different degrees of fermentation. In general, it appears that the loss of organic matter varied with the condition of the hay, being greatest for those samples which were charred or moldy. The loss for the brown hay appeared to be less than for the black, but in both cases it was much greater than would be expected for hay cured in the usual way.

From comparison of the samples of black alfalfa secured December 28 with those secured March 28 it appears that the loss of organic matter increased with the time the hay remained in the stack.

FEEDING VALUE

The feeding value of black alfalfa and brown alfalfa as compared with alfalfa cured in the usual way was determined by feeding each to steers which received a ration of shelled corn and oil meal in addition to the hay. The data secured in this feeding test were furnished by Dr. C. W. McCampbell, of the Department of Animal Husbandry.

Three lots of 14 steers each were fed 180 days. One of these lots was given black alfalfa represented by samples 591 and 592 in Table II, another was fed ordinary brown alfalfa hay that had been cured in the usual way, and a third lot was fed first quality alfalfa hay of good color. In all other respects the three lots were given the same feed. The data are given in Table V. In calculating costs and profits, the following prices for feeds were used:

Corn	per bushel	$1.12
Alfalfa hay	per ton	15.00
Black alfalfa	do	5.00
Brown alfalfa	do	15.00
Oil meal	do	45.00

The profits from feeding the ordinary brown alfalfa and the first quality green alfalfa hay were nearly the same, the difference being only $0.31 per steer. Also there was no essential difference in the daily gain made by the two lots.

The steers fed black alfalfa made unsatisfactory gains. There was a loss of $3.51 per head in spite of the fact that the black alfalfa was valued at only one-third as much as brown alfalfa or ordinary green alfalfa hay.

TABLE V.—*Steer-feeding experiment, 180 days, comparing black alfalfa with brown and green alfalfa hay*

Factors in experiment.	Lot 26, fed shelled corn, oil meal, alfalfa hay (good color).	Lot 27, fed shelled corn, oil meal, alfalfa hay (brown).	Lot 29, fed shelled corn, oil meal, alfalfa hay (black).
	Pounds.	*Pounds.*	*Pounds.*
Initial weight	334.5	334.7	338.6
Final weight	688.3	684.8	600.7
Total gain	353.8	350.1	262.1
Average daily gain	1.96	1.94	1.45
Average daily ration:			
Grain	7.39	7.39	6.65
Oil meal	.49	.49	.49
Alfalfa hay	7.82	7.82	[a]9.24
Total feed consumed:			
Shelled corn	1,330.2	1,330.2	1,197.00
Oil meal	88.2	88.2	88.2
Alfalfa hay	1,407.6	1,407.6	[a]1,663.5
Feed required to produce 100 pounds gain:			
Shelled corn	375.9	379.9	456.6
Oil meal	24.92	23.18	33.65
Alfalfa hay	397.8	402.01	[a]634.6
Cost of feed per day	$0.216	$0.216	$0.180
Cost of 100 pounds gain	11.01	11.10	12.36
Cost of feed per steer	38.88	38.88	32.40
Initial cost of steer at $8.50 per hundredweight	28.43	28.45	28.78
Total cost of steer	67.31	67.33	61.18
Final cost per hundredweight	9.77	9.83	10.18
Final value per hundredweight	10.25	10.35	9.60
Final value of steer	70.55	79.88	57.67
Profit per steer	3.24	3.55	−3.51

[a] Calculated to 8 per cent moisture basis to compare with alfalfa fed to other lots.

SUMMARY AND CONCLUSION

(1) Partially wilted alfalfa stacked without curing undergoes changes which result in the loss of about two-fifths of the organic matter.

(2) This loss apparently increases with the length of time in the stack and with the degree of fermentative changes that occur.

(3) Alfalfa which has become black as a result of fermentation is very inferior as a feed for steers in comparison with both brown alfalfa hay and alfalfa hay of good color and quality.

COTTON ROOTROT SPOTS

By C. S. Scofield, *Agriculturist in Charge, Western Irrigation Agriculture, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The disease of cotton commonly known as rootrot, which occurs in certain sections of Texas, New Mexico, and Arizona, usually appears in cotton fields during the latter part of the growing season. The affected plants wilt down rapidly and within a few days become dry and turn brown.

It is generally believed that this disease is due to a soil-inhabiting fungus known as *Ozonium omnivorum*,[1] which invades the root system of the host plant and by breaking down the root tissue cuts off the water supply and causes death. The same fungus is believed to attack many species of plants other than cotton, though the grasses appear to be immune.

One of the peculiarities of the rootrot disease as it occurs in cotton fields is that it usually appears in certain well-defined areas or spots within the limits of which nearly every cotton plant is killed. With the advance of the season, these spots of dead cotton gradually increase in size, the disease apparently spreading from plant to plant. Occasionally a plant remains alive within the infected area, but upon examination it is found that the lower roots are dead and that continued growth is supported by one or more lateral roots that branch out close to the surface of the soil.

The well-defined areal occurrence of the disease and the completeness with which it kills all the plants within the area naturally led to the impression that its destructiveness must be due to some purely local soil condition. Furthermore, it has been thought that the disease reappears from year to year in the same spots.

FIELD OBSERVATIONS[2]

Rootrot has been prevalent in the vicinity of San Antonio, Tex., for many years, and an opportunity has been afforded to observe its behavior at a field station located about 5 miles south of the city of San Antonio, where an extensive series of crop rotations have been conducted since 1909. The disease was so serious on the rotation plots of cotton in 1916 that it seemed advisable to survey each plot and locate definitely the infected areas, with a view to determining the rate of

[1] More recently named *Phymatotrichum omnivorum* (Shear) Duggar. (Duggar, B. M. The Texas rootrot fungus and its conidial stage. *In* Ann. Mo. Bot. Gard., v. 3, No. 1, p. 22. 1916.)

[2] The author is indebted to Mr. C. R. Letteer, Superintendent, and Mr. A. A. Bryan, Assistant, at the San Antonio Field Station for cooperation in making the observations here reported.

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spread in future years and also whether any of the different rotation and tillage treatments were really effective in retarding this spread.

These rotation plots are ¼ acre in size, being 264 feet long and 41.25 feet wide, and afford space for 10 rows of cotton 4.1 feet apart. Each row was carefully measured, and the location of the portion of the row in which the plants were dead was indicated on a diagram drawn to scale. The survey of 1916 was made near the end of the growing season, October 21, after the final picking of cotton had been finished. One of these plot diagrams, showing the areas of dead plants, is shown in figure 1. This diagram shows two main areas of infection, as shown by the brush-like lines. A count of the living and dead plants in this plot at the time the survey was made showed that 60.5 per cent of the total number of plants were dead. This plot had been planted in cotton each year since 1909. It had been plowed in November each year and had received an annual application of manure at the rate of 12 tons per acre.

The plowing and other tillage operations were made lengthwise of the plot so that any distribution of soil infection would naturally be favored.

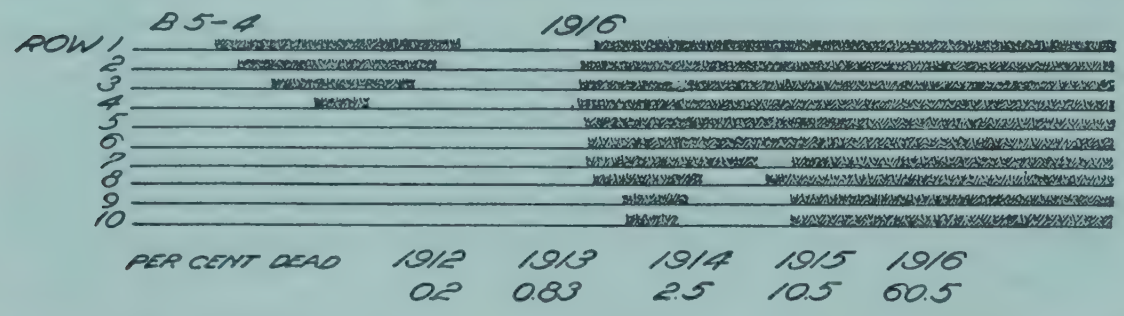

FIG. 1.—Diagram of plot B5-4, showing by the brushlike lines the portions of the rows in which the cotton plants were killed by rootrot in 1916.

Notwithstanding this fact, the limits of the affected areas were very sharp, dead plants standing adjacent to living ones in each row at the edge of the diseased area.

This same plot was planted to cotton again in 1917, each row being planted as nearly as possible in the same place as in the previous season. The count of living and dead plants and the diagram of the areas of dead plants were made on October 25, 1917, at the end of the growing season. The count of plants showed that 36.8 per cent of the total number were dead with symptoms of rootrot. The diagram of the plot for 1917 is shown in figure 2.

The distribution of the disease in 1917 was more scattered than in 1916; and the spot that is shown on the north side of the west half of the plot in the 1916 diagram (fig. 1) gives some indication of a progressive spread, in that living plants were found in 1917 where only dead plants were noted in 1916. This tendency for the spread of the disease to take place like the spreading of a fairy ring is not very pronounced, however, as can be seen in the diagram for the east half of the plot, nor is it to be found so definitely expressed in the diagrams of other plots.

The distribution of the diseased areas on this same plot in 1918 is shown in figure 3. The diagram for that year was made on October 28, 1918, when a count of the living and dead plants showed that 42.0 per cent of the total number were dead, apparently from the effect of rootrot.

The west half of this plot again showed some indication of a progressive spread of the disease, in that there was a V-shaped area of living plants in approximately the same place where nearly complete infection had been noted the previous season. The 1916 area of infection was

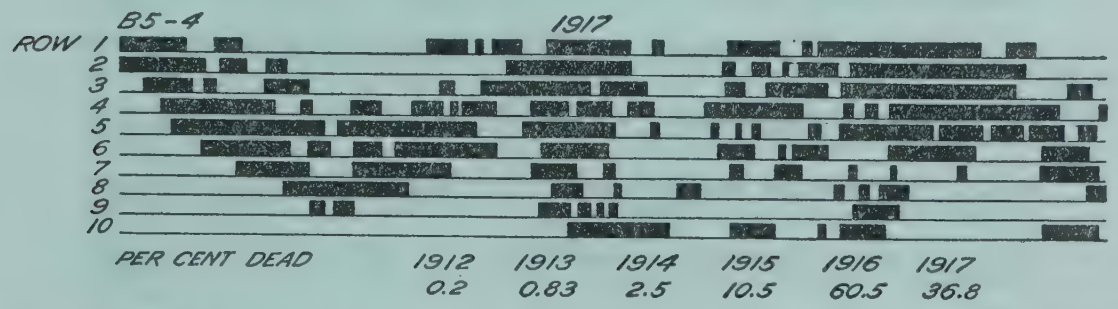

FIG. 2.—Diagram of plot B5-4, showing by the heavy lines the portions of the rows in which the cotton plants were killed by rootrot in 1917.

again infected, as were also the areas in the southwest corner of the plot and in the southeast corner of the west half, areas that had been free from dead plants in 1916 and 1917. However, this tendency toward alternate occurrence or progressive spread was not shown in the east end of the plot or in other plots with sufficient regularity to be considered significant.

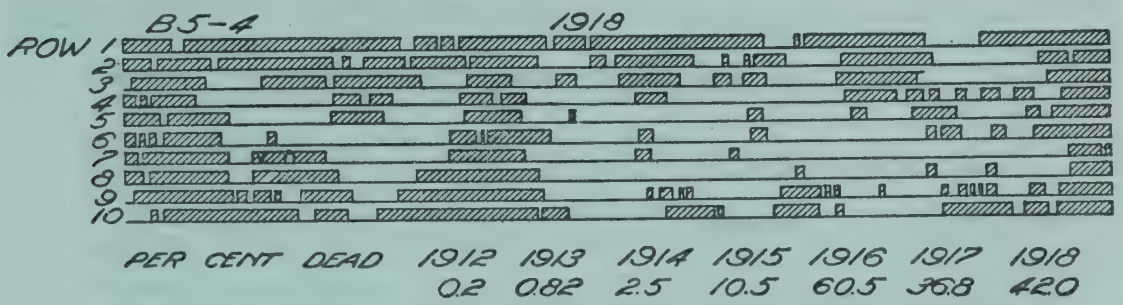

FIG. 3.—Diagram of plot B5-4, showing by the diagonal hatching the portions of the rows in which the cotton plants were killed by rootrot in 1918.

SUMMARY OF THREE YEARS' RECORDS

If the records of the occurrence of the disease in this plot for the three years be brought together as in figure 4, it will be seen that almost the entire plot has been affected within that time. Yet during the last two years less than half the plants have been taken by the disease.

It seems clear from the evidence here presented that these rootrot spots do not carry over from year to year. This may explain the difficulty that has been experienced by investigators in attempting to determine, by a comparative study of the soil conditions inside and outside the rootrot spots, what conditions permit the disease to become destructive.

There are three other plots in these San Antonio rotations on which cotton has been grown each year and on which the location of the diseased plants has been recorded since 1916. On plot A4–19 (fig. 5)

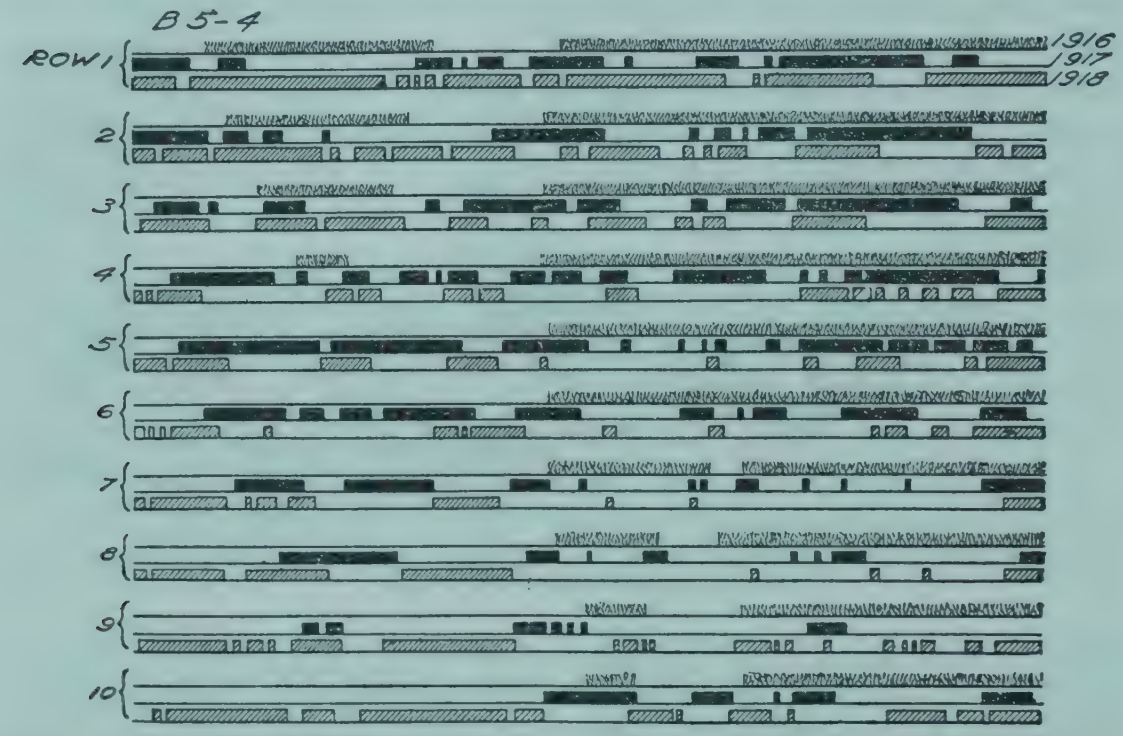

FIG. 4.—Diagram of plot B5–4, showing the portions of the rows in which the cotton plants were killed each year by rootrot in 1916, 1917, and 1918.

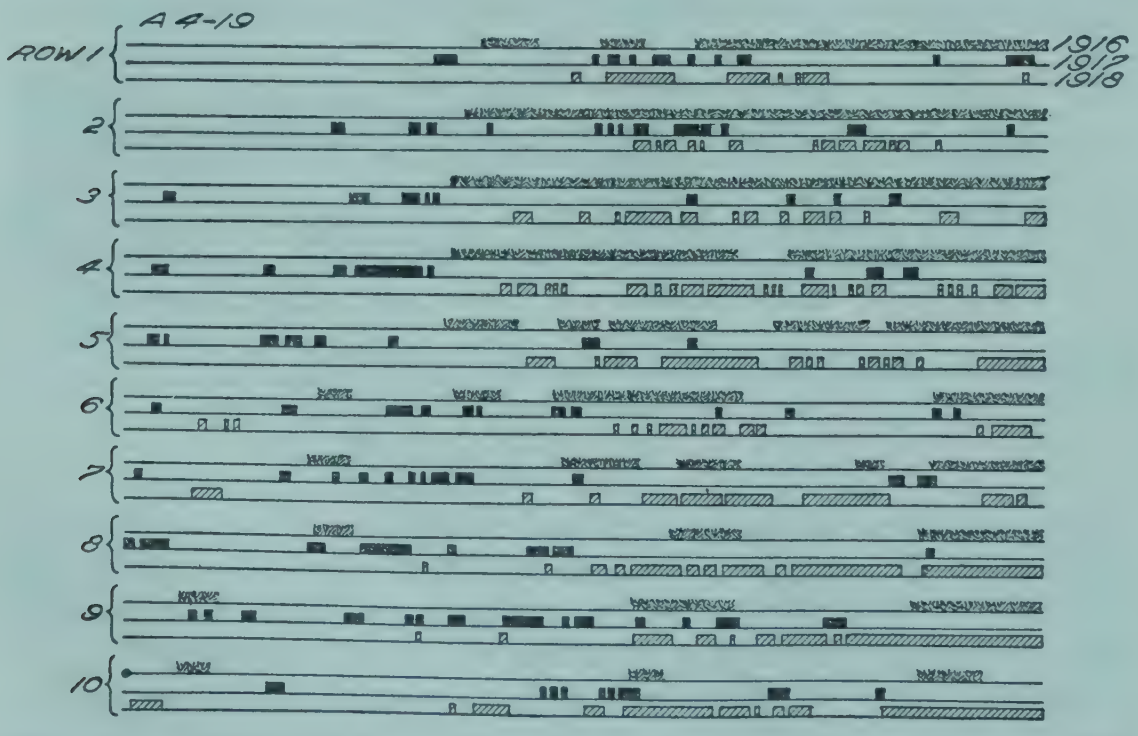

FIG. 5.—Diagram of plot A4–19, showing the portions of the rows in which the cotton plants were killed each year by rootrot in 1916, 1917, and 1918.

cotton has been grown each summer since 1913. The cotton stalks are plowed out in the fall, and the land is then disked and seeded to Canada field peas, which are plowed under the following spring in time to plant cotton

again. On plot A6–3 (fig. 6), cotton has been grown each summer since 1912, the land being plowed in the fall, after the cotton stalks are removed, and allowed to lie fallow during the winter. The plot B5–3

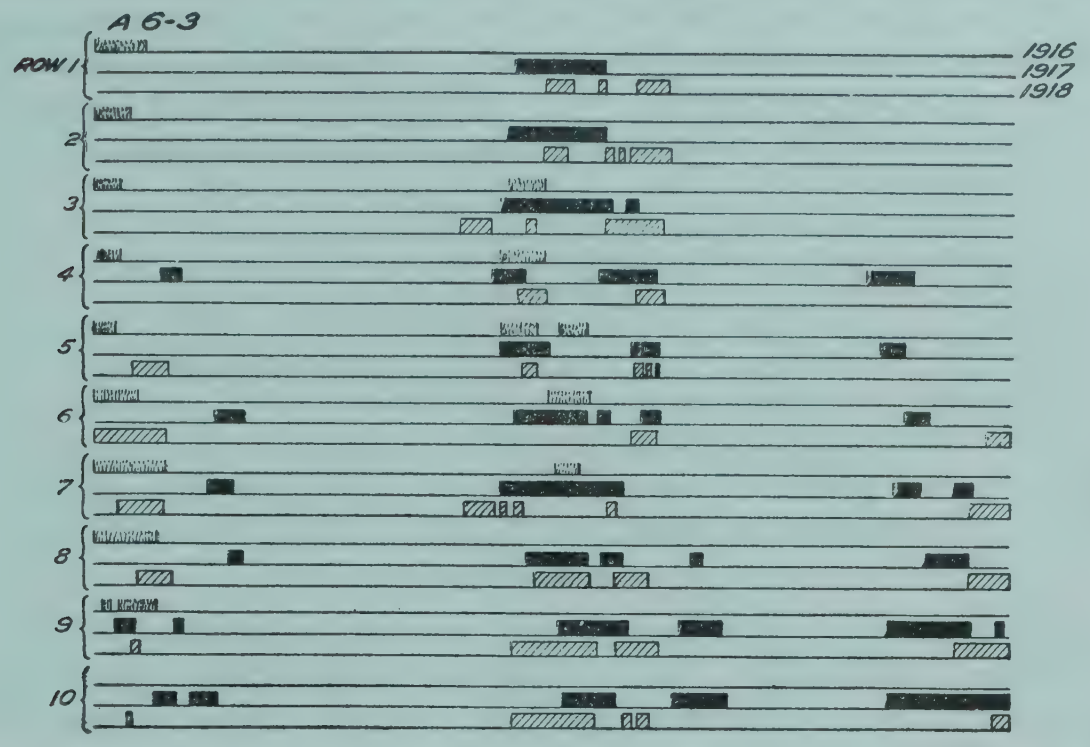

FIG. 6.—Diagram of plot A6–3, showing the portions of the rows in which the cotton plants were killed each year by rootrot in 1916, 1917, and 1918.

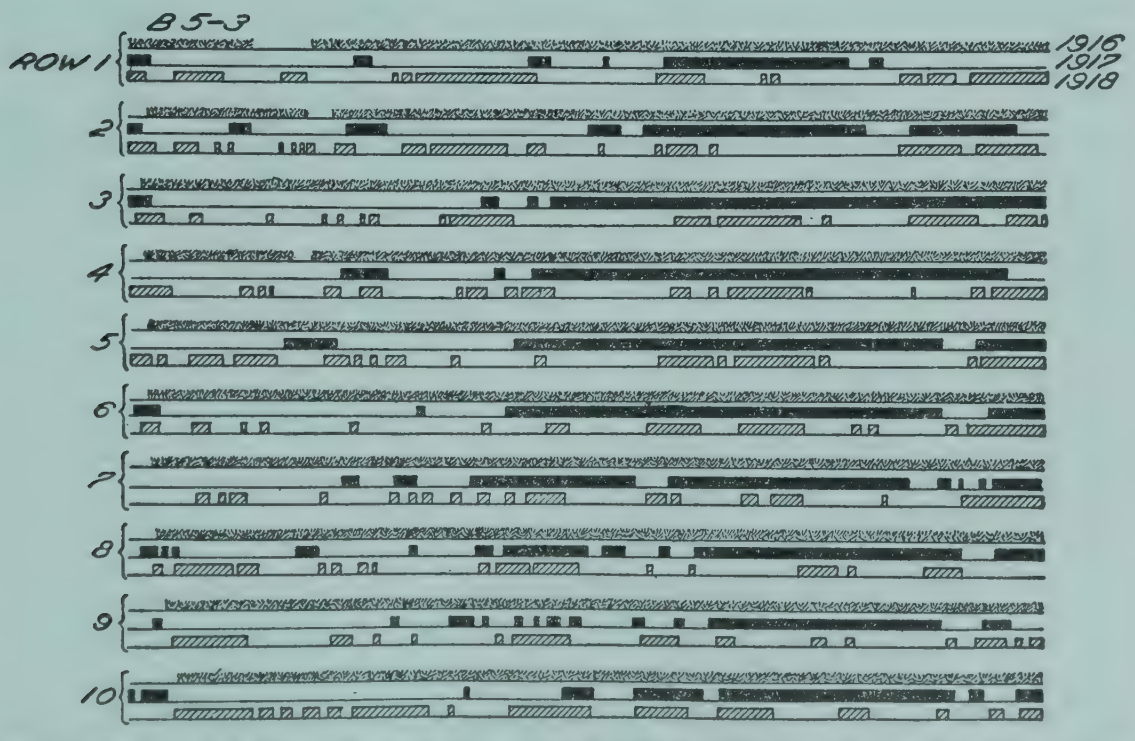

FIG. 7.—Diagram of plot B5–3, showing the portions of the rows in which the cotton plants were killed each year by rootrot in 1916, 1917, and 1918.

(fig. 7) receives the same cultural treatment as A6–3; and B5–4 receives the same treatment, except that manure is applied in the fall each year at the rate of 6 tons per acre. The diagrams of the occurrence of rootrot

in all these plots show conclusively that the disease does not continue to reappear in successive seasons in the same spots.

To complete the record of these field observations concerning cotton rootrot as it has been observed in these four plots, the percentages of loss for each season are shown in Table I. These percentages were determined at the end of the crop season by counting the total number of living and dead plants and dividing the number of dead plants by the total number of plants.

TABLE 1.—*Percentage of cotton plants taken by rootrot in rotation plots at San Antonio, Tex.*

Plot.	Treatment.	1912.	1913.	1914.	1915.	1916.	1917.	1918.
A4–19	Field peas; spring plowed		0.0	4.2	11.7	42.0	10.6	25.6
A6–3	Fall plowed	0.7	.82	.46	.7	7.4	15.1	9.2
B5–3	do	.9	3.8	17.6	49.4	96.2	43.7	30.3
B5–4	Fall plowed; manured	.2	.83	2.5	10.5	60.5	36.8	42.0

This table shows that there has been a marked increase since 1912 in the percentage of plants dying from rootrot, yet the disease has not been so severe in the last two seasons as it was in 1916. There does not appear to be a very direct or significant relation between the climatic conditions and the extent of the disease.

It is not the purpose here to attempt to explain the anomalous distribution of rootrot spots from year to year or to suggest any cultural method for the control of the disease. It is rather to show that even though the disease does usually occur in well-defined spots in one season, it may not recur there the following season but may appear in a new place.

APPLE-GRAIN APHIS

By A. C. BAKER, *Entomologist*, and W. F. TURNER,[1] *Entomological Assistant, Deciduous Fruit Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

The present paper is a brief report of the life-history studies of the apple-grain aphis, or "apple-bud aphis," made at Vienna, Va., during 1914–1916. The species studied is perhaps the most abundant, though not the most important spring form occurring on apples. At the time the studies were undertaken two theories were set forth in the literature in regard to the life history of this aphid. Some authors claimed an annual migration, while others claimed a possible biennial one. At that time also the species was known as *Aphis avenae* Fab., but systematic studies based on the Fitch types and European material made in connection with the biological work show that it should be called *Rhopalosiphum prunifoliae* (Fitch).

Sufficient work has been completed to indicate that a number of species, very similar in their summer generations on grains, have in the past been confused. Some of these are as follows:

I. *Rhopalosiphum prunifoliae* (Fitch), the subject of this paper, which winters upon the apple and migrates to grains and grasses. This is the form which has been incorrectly called *Aphis avenae* Fab.

II. *Rhopalosiphum padi* (L.). This species is abundant in Europe upon the bird cherry and migrates to grasses in the summer. *Aphis avenae* Fab. is a synonym, and probably also *A. pseudoavenae* Patch.

III. *Aphis cerasifoliae* Fitch. This species is a common form on our choke-cherries, and in its summer forms on grass is almost indistinguishable from the two former species.

A number of other species very similar in their summer forms occur, but either these are undescribed or their life histories are not fully worked out.

LIFE HISTORY OF *RHOPALOSIPHUM PRUNIFOLIAE* (FITCH)

EGG

LOCATION ON TREE

The eggs of this species are to be found mainly on the small branches of the lower portions of the trees. In heavy infestations they may be found in similar locations all over the trees. In the winter of 1915–16 eggs were found at Vienna, in the very tops of old apple trees 40 feet

[1] Resigned Mar. 7, 1916.

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high. Occasionally, also, eggs of this species will be found on watersprouts; but this is not one of the favorite positions.

The eggs are deposited between the fruit buds on the twigs, in the little impressions occurring on the fruit spurs, in scars, etc., and even exposed on the twigs and small branches. On small trees they may be found occasionally in crevices of the bark on the trunk and main branches.

HATCHING

During the spring of 1915 eggs began hatching at Arlington Farm, Va., about March 15. On March 16 a few scattered young were found on the trees. At Vienna, Va., hatching began about March 27. A few eggs hatched earlier than this, but these were so rare that they need not be considered. In fact all the young which emerged before April 3 at Vienna died during a period of cold weather occurring from March 29 to April 2.

The important hatching began, therefore, on April 3 and continued till April 10. It was most rapid from April 5 to 7, nearly all the eggs having hatched before the eighth.

In connection with the early hatching which we have noted, it should be borne in mind that eggs of this species may hatch at any time during the winter, at least after about the first of January, in case warm temperatures prevail for a period of four or five days. Such hatching frequently occurs in the vicinity of Washington.

STEM MOTHER

LENGTH OF NYMPHAL LIFE

The average duration of the nymphal period was 13 days, 11 different insects becoming adult just 13 days after hatching. A few attained the adult condition in 12 days, while others required 14. The length of the different instars varied considerably, this being due apparently entirely to temperature conditions. In all cases under observation a long period in one stage was compensated for by a short period in the following instar. In general the first instar was longest, requiring 4 or 5 days; the second was shorter, 2 or 3 days; the third and fourth were about equal, being between 3 and 4 days each.

TOTAL LENGTH OF LIFE AND DURATION OF STEM-MOTHER PERIOD

The total length of life of stem mothers varied from 23 to over 49 days, the average for seven insects being 38.4+. The duration of the generation in 1915 was from April 3 to May 26. It should be noted that while some eggs hatched as early as March 24 all the insects born before April 3 were killed by freezing temperatures.

REPRODUCTION

The average number of young produced by seven stem mothers was 99, in an average of slightly less than 19 days. The greatest number produced by one insect was 131 in 20 days; the least was 51 young produced in 7 days, an average production of 7.27 young per day. This was the greatest average daily production by any one stem mother.

The actual number produced per day varied from none to 16. There was no uniformity in the frequency of the births, some insects depositing large numbers in a short period and resting for some time between these periods, others depositing a few each day.

One insect gave birth to 22 young, all of which were deposited with unruptured membranes. None of these insects lived more than 24 hours. None of the other mothers produced young in this condition.

A second adult produced 80 young in 10 days.[1] She was injured on the eleventh day, her abdomen being slightly crushed. While she lived for 14 days after the accident and appeared perfectly normal, she failed to produce any more young. The abdomen attained its normal shape within 24 hours, and the insect fed normally.

In most cases the abdomen began to shrink toward the end of the reproductive period, and the mother lived but one or two days after producing her last young. In fact some died almost immediately. In two cases, however, no shrinking was apparent. It was noticed that toward the end of the reproductive period the abdomens of these two insects were becoming darker and irregularly mottled. One of these insects lived for 13 days after producing her last young, while the other was fixed for sectioning after a period of 17 days. Both continued feeding throughout the period, and neither showed the least sign of shrinking. When the preserved specimen was sectioned, it was found that the reproductive system had almost entirely disintegrated. Three embryos were present, but these also were disintegrating. The digestive tract was apparently normal. The abdomen was almost entirely filled by the fat body, which extended well into the thorax. The injured specimen which has been mentioned suffered the same color changes that these two insects did, and although no examination was made it seems possible that the conditions occurring in these two insects were produced artificially in the third aphid through injury.

FEEDING HABITS

During the spring of 1915, as has been stated, some eggs hatched as early as March 15. At this time the apple buds had not begun to swell. These early individuals were all killed shortly after emergence by a return of low temperatures, and it was impossible to determine whether or not they might have lived on the unopened buds.

[1] This average of eight young per day was not considered in obtaining the figures given, since the insect did not complete her reproduction period normally.

By April 4, the earliest date at which insects hatched and lived to become adults, the buds had commenced to swell, and many of them showed a little green. Within two or three days the majority of them had begun to open, providing an abundance of food for the young stem mothers. These could be found clustered on the terminal buds, which open earliest, frequently well down between the very small leaves.

By the time the stem mothers had become adults many of the buds were entirely open and their leaves had completely expanded. The stem mothers almost invariably located themselves upon the petioles of such leaves, head downward. The young migrated, almost immediately after birth, to the under surface of the leaf, where a cluster of them was soon formed.

SPRING FORMS

PERCENTAGE OF ALATE FORMS

During the season of 1915, four generations of this species (after the stem mother) were bred on apple. Migrants appeared in all generations. Of 101 aphids reared to maturity in the second generation 89.1 per cent were alate. In the third generation, of 34 insects 58.4 per cent were alate.[1] In the fourth generation 98.5 per cent were alate, only 1 insect out of 67 being apterous. All young from this single apterous aphid of the fourth generation bore wings, and during the season of 1914 all insects of the fourth generation were winged. This seems to be the normal condition.

SPRING APTEROUS FORM ON APPLE

LENGTH OF NYMPHAL LIFE

The average duration of the nymphal period of the spring apterous form was eight days, varying from seven to nine. Three insects born on April 20 became adults in seven days. The mean temperature for the period was 66.3° F. Three other insects, born on April 28, required nine days to reach maturity with a mean temperature of 61.4°. Both lots of insects averaged about six days for the first three months. The mean temperatures for this period were 65° for the early brood and 62° for the later. Moreover, this difference in mean temperature was due almost wholly to the temperatures obtaining on the sixth or last day, when temperature has the least effect on the stages in question.

REPRODUCTION

Complete records of reproduction for the spring apterous form were obtained with only six individuals. The average reproduction of these six was 73.5 young. The greatest number of progeny from one mother was 88, while the smallest number was 51. The average duration of the

[1] The reason for the high percentage of wingless insects in this generation is that only a few were reared, and of nine young from one mother six became apterous.

reproduction period was 15.3 days, varying between 10 and 23. The greatest average daily production was 5.8 young and the lowest 3.39, the daily average for the six insects being 4.44.

TOTAL LENGTH OF LIFE

One insect of the spring apterous form lived for 42 days. The average length of life, however, was only 30.8 days, and two aphids died when only 21 days old.

SPRING MIGRANT

LENGTH OF NYMPHAL LIFE

This period varied from 8 to 12 days, the average for 22 insects being 8.36 days. The variation in this period was closely connected with the variation in mean temperatures. The time occupied by the nymphal stage was divided rather evenly between the four instars concerned, the final instar being a little longer than the others. The final instar of the spring migrants was usually about one day longer than that of the apterous insects born the same day.

SPRING MIGRATION

In 1915, at Vienna, Va., migration began about May 1 and continued till about June 7. No general migration occurred with this species either in 1914 or 1915. The alate insects left the apple singly a day or two after having become adults. Migrants could not be found on grasses in any abundance, though single individuals were taken here and there in the fields. No attempt was made to determine the species of Graminaceae upon which this insect spends the summer. In the experiments it lived easily on both oats and wheat, the former being used more generally because it was found to be easier to handle.

REPRODUCTION

Complete data covering reproduction were obtained with 21 insects. The average number of young produced per mother was 13.5. The smallest number produced by one insect was 9 and the largest 20. Ten insects produced from 9 to 13 young each. The average daily production was 0.85 per mother. These spring migrants produced very irregularly, often passing as much as 3 or 4 days without giving birth to any young. The usual number produced on one day, taking into account only the days during which reproduction actually occurred, was about 3. One insect produced 10 young within 24 hours.

TOTAL LENGTH OF LIFE

The average total length of life for the spring migrant was a little over 27.6 days. The shortest for one insect was 15 days. All other insects lived over 20 days. One was fixed after having lived for 37 days.

SUMMER FORMS

The great majority of the insects living upon oats during the summer are apterous. During the early part of the season, however, a considerable number of alate insects were reared. These occurred in all generations from the second to the twelfth of the summer forms and the fourth to the sixteenth[1] from the egg. It should be noted, however, that the general distribution of the summer alate form was limited to the first seven summer generations. Such forms were limited to four lines after that period, and in three of these four lines the form occurred only once. In the other line alate individuals, or at least forms other than apterous, occurred in five consecutive generations after the appearance of such forms had ceased in practically all other lines. In all cases these were the progeny of apterous mothers, no attempt being made to rear young from the alate insects. This might seem to indicate that the alate form was an inheritable character, for this particular line at least. The appearance of this insect in this series of experiments can hardly be traced to food conditions, since the food was changed two or three times in the course of the five generations. Strong negative proof is also furnished by the fact that in other lines the food was so poor at times that we had difficulty in maintaining the insects, yet no unusual number of alate aphids appeared and, in fact, in many cases no winged forms at all developed. It should be added that the percentage of the alate insects occurring in the five generations under discussion was small.

In addition to the two common forms, intermediates occurred in five experiments. In four experiments they were accompanied by both apterous and alate forms, as was found by the authors to be the case in *Aphis pomi*.[2] In the fifth experiment no alate forms were obtained. Several pupæ were lost in this case, however; and since only 2 or 3 intermediates developed from 10 or 12 pupæ in the other experiments, it appears quite probable that some of these lost pupæ would have developed into alate forms.

FEEDING HABITS

On the oats and wheat the insects locate mainly on the stems and on the lower portions of the leaves. Only small plants could be used in the experiments; and an occasional insect might be found feeding on any portion of the plant, except that little tendency to locate at or below the surface of the ground was noted during the season of 1915. It may be, however, that during hot, dry seasons the insects would prefer such locations.

[1] This apparent discrepancy is due to the fact that late alate forms occurred only in those lines descended from spring migrants of the fourth generation.

[2] BAKER, A. C., and TURNER, W. F. MORPHOLOGY AND BIOLOGY OF THE GREEN APPLE APHIS. *In* Jour. Agr. Research, v. 5, no. 21, p. 955–994, 4 fig., pl. 67–75. 1916.

MIGRATION

Summer migration, of course, is carried on mainly by the alate insects. The apterous mothers, however, frequently wander from plant to plant. They seldom deposit more than two or three young without changing their position, and frequently the young from one mother will be found on four or five different plants. In one case a mother deposited young on four different plants in less than 48 hours.

The young themselves, by the time they are two or three days old, wander considerably. Usually they do not leave the plant on which they were born, but occasionally they will. This is especially true in case such plants furnish insufficient nourishment.

As was found to be true with *Aphis pomi*,[1] the alate insects occurring during the summer evinced no particular tendency to leave the plants on which they developed. Occasionally one was found wandering about on the inner surface of the cage, but usually they remained on the plants, fed, and reproduced without any more restlessness, certainly, than was shown by the apterous insects.

SUMMER APTEROUS FORM

DURATION OF LARVAL STAGES

The immature stages of the summer apterous form covered periods varying from 6 to 12 days, the length of the period being almost wholly controlled by temperature conditions. Out of several hundred experiments with this form, evidence of an effect on the duration of the nymphal period could be traced to food conditions in only two cases. In both of these experiments the young aphids were living on very poor plants, and in both they required about 2 more days to attain maturity than did the insects born on the same day in other experiments.

The chart in figure 1 shows how important the effect of the temperature is upon the length of the larval period. The figures for temperature read down and those for number of days read up, since the length of the period varies inversely with some factor of the temperature, and this method allows the curves to parallel instead of cross and so facilitates the reading of the chart. It must be remembered that this chart shows the temperature effect only in a general way, since the length of the periods, while listed in whole number of days, actually varies from the number by a few or possibly many hours in many cases. Since observations were made only once a day it is impossible to give more accurate figures than are given here. Moreover, in certain individual cases in which the mean temperatures varied greatly during a given period, the retardation caused by low temperatures during one period of the life of the immature aphid was found to be not wholly compensated for by

[1] Baker, A. C., and Turner, W. F. op. cit.

acceleration in growth caused by later, higher temperatures, or vice versa. Nevertheless the effect of the temperature was so marked that it was felt to be worth while to call attention to it in this manner.

In this connection it might be well to note the fact that the season of 1915, at Vienna, Va., was an excellent one for the rearing of aphids, since the summer was cool throughout and the humidity was high. Results obtained during other years seem to indicate that excessively high temperatures, at least when accompanied by dry weather, may have a direct effect, rather than an inverse one, upon the length of this period. It may be, however, that such effects are due to excessive drought rather than to high temperatures.

REPRODUCTION

The average number of young produced by 102 insects was 28.1 each. The insects used in obtaining these figures were secured from all the

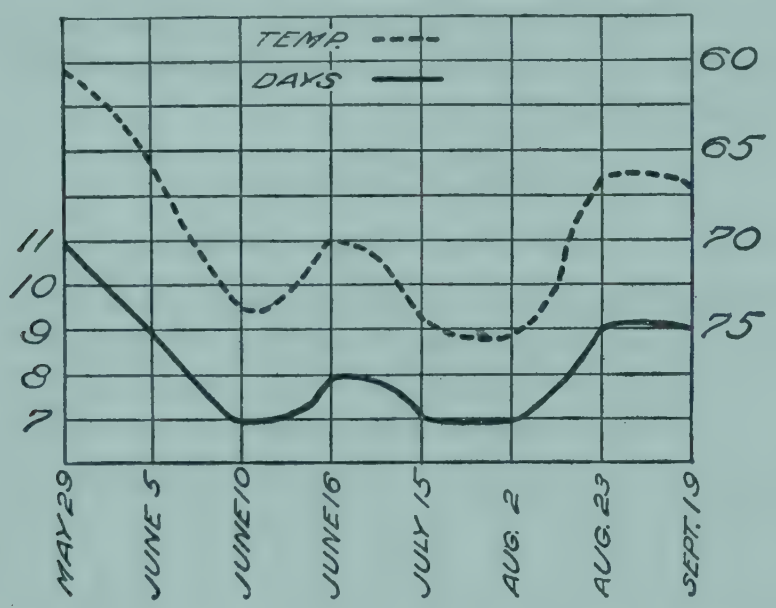

FIG. 1.—Effect of temperature upon the duration of the larval stages of the apple-grain aphis.

summer generations. No difference was observed in this matter between mothers occurring in the early summer, the middle of summer, and the early fall. The average reproductive period for this insect was 17.9 days, giving an average of a little over 1.5 young per day for the entire 102 insects.

This daily production varied somewhat with the season. The mothers producing young during late September and October produced an average of one or even less per day. Their reproductive period was considerably longer, however, so that the total number of young produced by one mother was 54, and the smallest was 12. The greatest daily average was 3 young, the mother producing 54 young in 18 days. This insect and one produced by the same mother also hold the record for greatest single daily production, the two together producing 41 young in 2 days.

LONGEVITY

The average length of life for 98 insects was a little over 28 days. In general the mothers died on the day on which they produced their last young. Toward the end of the season, however, several insects lived for a period of from four to nine days after reproduction had ceased. This was apparently due, in part at least, to the fact that reproduction was not so rapid during this period as it was earlier in the summer and the mothers were not completely exhausted as were the others.

SUMMER INTERMEDIATE FORM

As has been stated previously, the summer intermediate form occurred in only five experiments. Young were reared from only two mothers, and these were both preserved for microscopical examination before they had completed their reproductive period. From our meager records it appears that the length of nymphal life is equal to that of the alate form. Such is the case, at least, in the five experiments under consideration. On the other hand, the reproductive activities appear to be patterned after those of the apterous aphids. One of the intermediates produced 17 young in six days and was then preserved. The body still contained numerous embryos, indicating that normally it might have produced as many young as the average, at least, of the apterous insects.

SUMMER ALATE FORM

LENGTH OF IMMATURE STAGES

Like the spring migrant, the summer alate form required from 1½ to 2 days more for the attainment of the adult form than did the apterous aphids. The record for 14 insects gives a period varying from 9 to 12 days. In each case this was about 2 days longer than was required by apterous insects in the same experiment.

REPRODUCTION

Complete records of the reproductive activities of this form were obtained for only 8 insects. These 8 produced an average of 16.25 young per mother. The greatest number produced by one insect was 23, while the smallest was 12. The average length of the reproductive period was 11.9 days, varying from 7 to 20; and the average daily production for the 8 insects was 1.36 young per mother per day.

LONGEVITY

The average length of life of the 8 insects just mentioned was 22.2 days. In all but 2 cases the mothers died upon the day on which they produced their last young. These 2 insects each lived for 2 days after reproduction ceased.

FALL FORMS

PRODUCTION

In this species only the apterous summer vivipara produced the fall forms, no alate insects occurring in the experiments at a late enough period for such production.

One mother may produce fall migrants (alate sexupara) and males, apterous vivipara and fall migrants, or apterous vivipara and males. In two cases at least it seems very probable that one female produced all three forms, but this cannot be stated with absolute certainty.

Apterous vivipara and fall migrants were produced promiscuously, just as were apterous and alate summer vivipara earlier in the season. On the other hand, when males were produced they were the last progeny of their mother, while the other form, whether it was the apterous vivipara form or fall migrant, constituted the earlier progeny. In this manner, the males, while produced in the same generation with the fall migrants, matured at about the same time as did the ovipara (progeny of the fall migrants). In no case can it be certainly stated that one mother produced only fall migrants; on the other hand, all of the progeny raised from some of the apterous sisters of fall migrants were males.

FALL MIGRANT

MIGRATION

As was the case with spring migrants, the actual migration of this form did not occur as a swarming but was a scattered affair in the field, covering a period of from three to four weeks. This would be expected since the fall migrants did not all occur in one generation but in several. The insects may remain on the summer host for one or two days after becoming adult; and they may spend an equal, or in a very few cases even a longer period on the apple before reproduction begins.

During the fall of 1915 the apple-grain aphis was very abundant at Vienna. After migration had been in progress for two or three weeks, an examination of several old trees on the laboratory grounds revealed the presence of migrants and their progeny on practically every leaf. Almost no migrants were observed on the twigs, and those few were wandering about. In the fall of 1914 these insects were not very abundant, and only scattered migrants could be found on the trees. In so far as could be determined no choice was shown in the selection of leaves. Some insects were on leaves near the side branches and still others on the watersprouts. Frequently, however, even in light infestations, the insects would be found in groups of three or four on scattered leaves rather than singly.

DURATION OF LARVAL STAGE

Fall migrants required from 14 to 17 days to attain maturity, this being three or four days more than was required by their apterous sisters. It is probable that the increased length of this period is due almost entirely to the lower temperatures prevailing in the fall, this accounting not only for the lengthening of the immature period of the apterous insects but also for the further lagging of the alate form behind the apterous. However, the fact that the fall migrant is much larger than the apterous form (and than the summer migrant) may have an effect.

REPRODUCTION

Sixteen fall migrants produced an average of 5.9 young apiece, the number varying from four to eight. In every case the mothers produced all their young within a period of from 24 to 36 hours. In other words, the mother produced them rapidly with no long resting period between births.

LONGEVITY

The average total length of life of seven fall migrants was a little over 33½ days. All the insects lived for some time after having finished reproduction, sometimes for as long as three weeks or more. One insect lived for 26 days after reproduction had been completed, its total length of life being 51 days. This short reproductive period followed by a prolonged life of several days was found to prevail also among the fall migrants of *Anuraphis malifoliae* Fitch.

OVIPARA

LENGTH OF LARVAL STAGES

The oviparous form matured very slowly in comparison with earlier forms of the year, requiring a period of from 16 to 20 days to reach maturity. It developed very irregularly, the amount of time spent in the several instars not being at all uniform for the various insects. This apparently was due to the fact that during especially cold periods the insects became quiescent, and since no development could take place during such a period the particular stage in which the insect was at the time would be prolonged.

MALE

DURATION OF LARVAL STAGES

In our experiments all males required about 16 days to attain maturity. This about equals the length of time required by fall migrants born late in the season.

MIGRATION

The males leave the summer host soon after becoming mature and fly to the apple trees. The earlier males usually arrive before the ovipara are mature. In such cases they usually wander about on the trees somewhat and then frequently settle down on the leaves for short periods at least and feed.

SEXES

MATING

As soon as the ovipara become adults they usually wander from the leaves to the twigs. The males also leave the foliage; and nearly all the mating occurs upon the twigs, although occasionally couples in copula can be found upon the leaves.

The males do not appear to have any special means for locating the ovipara. They wander restlessly about, and if they meet a female immediately try to mate with her. Sometimes the female will remain quiet for a few moments and at others the male is obliged to climb on her back while she is walking. Frequently a female will wander about with the male on her back throughout the entire process, which usually lasts from 10 to 15 minutes or even a little longer.

As soon as the male leaves the female the latter recommences wandering, in case she had stopped, till she finally locates a position for oviposition which suits her. The male in the meantime immediately starts searching for another female. A female also may mate at least twice before laying any eggs, though one mating seems to be sufficient for fertilization. It seems to be due simply to the fact that even if she has mated she makes no attempt to get away from any other male which she may meet, and consequently mating occurs again. Males, so far as our observations go, are always present in smaller numbers than are the females.

REPRODUCTION

Having selected a place for oviposition the female usually settles down and feeds for some time, occasionally even for several hours, before laying her first egg. Sometimes after laying this she remains in about the same position for a time and then deposits another. Usually, however, the insect will wander about considerably between the acts of oviposition, selecting a different location for each egg. A second female, however, may deposit an egg beside that already laid by the first aphid. In fact, in particularly favorable positions about buds, in injuries to the bark, etc., several eggs may be found and often several females may be seen packed closely together, all ovipositing at the same time. Occasionally the ovipara will wander back to the leaves and feed for short periods, soon returning to the twigs to continue oviposition.

LONGEVITY

In many cases, and apparently in most, the female dies shortly after depositing her last egg. The duration of the period of oviposition varies greatly with different insects, however, and in some cases the females are obliged to wait for some little time after becoming mature before males find them and mating takes place. Consequently the total length of life varies greatly with different individuals. The minimum, however, is about 20 days; this is for females which deposit only two or three eggs. The maximum can not be given accurately, since those ovipara which lived the longest in the experiments continued on the plants till December. Such insects during particularly cold weather became quiescent for several days at times and frequently did not oviposit for several days at a time, even during intervals of warm weather. Frequently some insects were killed by cold weather while others, as old or even older, survived. The life of this form, therefore, depends greatly upon whether it is born early or late, and upon the general temperature conditions prevailing during the season.

The males are about as long-lived as the females, omitting the exceptions just noted among the latter. The principal place of variation among individual males is the period from migration to mating, those insects which are obliged to wait for the females to mature usually living somewhat longer than the others.

OVIPOSITION

The greatest number of eggs produced by one mother in the experiments was seven. Others produced from one to three eggs. The custom of dissecting ovipara and deriving the number of eggs from the number of immature ova seems unsafe for aphids, since in several species it has been proved that some such ova do not become fully developed eggs but disintegrate within the bodies of the ovipara. From the observations made by the writers, therefore, it can be stated only that this species may lay as many as seven eggs.

FEEDING OF OVIPARA

As has been previously stated, the fall migrants settle upon the leaves of apple trees and produce their progeny there. From the results obtained in our experiments it seems that, while the stem mothers and spring forms on apple prefer tender, succulent foliage from which to obtain their food, the ovipara need hard, matured leaves. Most of the trees used in our experiments were taken from the field about three or four weeks before they were used for food. The old leaves dropped off in nearly every case, causing some of the buds to develop and produce small, succulent foliage such as the spring forms fed upon. In most cases

we had no difficulty in maintaining fall migrants on such plants, and these sexupara produced normally. The young ovipara, however, fed for from two to five days and then died. It was finally found necessary to transfer all the material to plants which had not shed their matured leaves, and on these there was no difficulty in raising the ovipara.

Under natural conditions it would be impossible for ovipara to obtain other food than that furnished by the mature leaves. It seems quite possible that the form having become adapted to such a source of food should be unable to maintain the proper balance when furnished with a source from which food may be obtained too rapidly or abundantly and consequently could not live.

We have been unable to find any foliage which, if still green, is too hard or dry for the insects to live upon.

SUNFLOWER SILAGE

By RAY E. NEIDIG and LULU E. VANCE, *Chemical Department, Idaho Agricultural Experiment Station*

In many sections of the Pacific Northwest the selection of a suitable crop for silage purposes is a matter of some difficulty because of the variable climatic conditions. New crops that are more or less resistant to drought and that will yield a heavy tonnage of green material per acre are greatly desired for this purpose. The results obtained by Arnett and Tretsven in 1917[1] on sunflower silage were so encouraging that the Idaho Experiment Station grew a plot of two acres for silage. During the early part of September, 1918, the sunflowers were cut and made into silage. This silage afforded an excellent opportunity for a chemical study of the acid formation. Since the kind of acid produced in silage is a criterion of the quality of silage, the results obtained would definitely establish the type of fermentation occurring when sunflowers are properly siloed.

The crop of sunflowers was not sufficient to fill the silo entirely, so corn was added in sufficient quantity. About 10 days elapsed between the siloing of the sunflowers and corn. The samples of silage were taken on January 9, 18, and 22 at the depth of 2, 6, and 9 feet from the top of the sunflower silage. Both volatile and nonvolatile acids were determined. Since the methods used have been previously described by one of the writers,[2] no detailed description will be given here. The Duclaux method was used to determine the volatile acids. In the calculations of our results, the Duclaux constants were used. Calculations were made by both the algebraic and the graphic methods suggested by Gillespie and Walters.[3] These methods greatly simplify the calculations when two or three acids are present. Lactic acid was determined as zinc lactate.

Sample 1 was taken from the silo on January 9 at a depth of 2 feet. The silage was dark in color and had a strong, disagreeable odor. Sample 2, taken on January 18 at a depth of 6 feet, was lighter in color, with only a slight disagreeable odor. Sample 3 was taken on January 22 at a depth of 9 feet. The color and odor of this silage were very good, and it appeared to have undergone normal silage fermentation.

The data on acidity of the three samples follow:

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[1] ARNETT, C. N., and TRETSVEN, Oscar. SUNFLOWER SILAGE FOR DAIRY COWS, PRELIMINARY REPORT. Mont. Agr. Exp. Sta. Bul. 118, p. 75–80. 1917.

[2] NEIDIG, Ray E. ACIDITY OF SILAGE MADE FROM VARIOUS CROPS. *In* Jour. Agr. Research, v. 14, no 10, p. 395–409. 1918.

[3] GILLESPIE, L. Y., and WALTERS, E. H. THE POSSIBILITIES AND LIMITATIONS OF THE DUCLAUX METHOD FOR THE ESTIMATION OF VOLATILE ACIDS. *In* Jour. Amer. Chem. Soc., v. 39, no. 9, p. 2027–2055, 3 fig. 1917. Literature cited, p. 2055.

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TABLE I.—*Acidity of sunflower silage, Idaho, 1919*

Sample No.	Date of sampling.	Distance from top of sunflower silage.	Moisture.	Dry material.	Acids in 100 gm. silage juice.						Acids in 100 gm. silage containing moisture.						Acids in 100 gm. silage on dry basis.					
					Acetic.	Propionic.	Butyric.	Total volatile acids.	Lactic.	Total acids.	Acetic.	Propionic.	Butyric.	Total volatile acids.	Lactic.	Total acids.	Acetic.	Propionic.	Butyric.	Total volatile acids.	Lactic.	Total acids.
		Ft.	*P.ct*	*P.ct*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	Jan. 9	2	76.6	23.4	0.54	0.00	0.96	1.50	(1)	1.50	0.41	0.00	0.74	1.15	(1)	1.15	1.77	0.00	3.14	4.91	(1)	4.91
2	18	6	78.0	22.0	.32	.07	.05	.44	0.43	.87	.25	.06	.04	.35	0.34	.69	1.13	.25	.18	1.56	1.54	3.10
3	22	9	81.0	19.0	.80	.09	.00	.89	.81	1.70	.65	.07	.00	72	.65	1.37	3.41	.38	.00	3.79	3.45	7.24

[1] Trace.

DISCUSSION OF RESULTS

Table I shows the kind and amount of acids found in the three samples of sunflower silage. The acid fermentation of sample 1 does not appear to be normal, for butyric acid is present in a large quantity while only a trace of lactic acid is found. Sample 2 can not be classed as first-grade silage, owing to the presence of butyric acid. Sample 3, however, at a depth of 9 feet showed an acid fermentation similar to that found in good corn silage, which is considered a typical fermentation. The abnormal fermentation of samples 1 and 2 is no doubt due to the fact that 10 days elapsed between the filling of the silo with sunflowers and the completion of the filling with corn. This period of time allowed considerable air to permeate the sunflower silage and favored the growth of organisms that are responsible for an anbormal fermentation. The results on sample 3 show that under the proper conditions sunflowers will produce an excellent grade of silage.

COMPOSITION OF SUNFLOWER SILAGE

Approximate analyses were made on the three samples. The results are given in Table II. The average of these results, together with the average of the results on 112 analyses of corn silage as given by Henry and Morrison,[1] follows:

[1] HENRY W. A., and MORRISON, F. B. FEEDS AND FEEDING. ed. 15, p. 645. Madison, Wis., 1915.

TABLE II.—*Comparison of the composition of sunflower and corn silage*

Kind of silage.	Water.	Ash.	Protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
Sunflower, sample number:	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	76.6	2.4	2.3	7.1	10.5	1.1
2	78.0	2.7	2.6	5.6	10.1	1.1
3	81.0	2.2	2.4	4.6	8.9	1.1
Average	78.5	2.4	2.4	5.8	9.8	1.1
Corn (average of 121 analyses)	73.7	1.7	2.1	6.3	15.4	0.8

The composition of sunflower silage compares very favorably with that of corn silage. No data are at present available on the digestion coefficients of sunflower silage, but it is hoped that such data will be secured during the coming year. Practical feeding, however, indicates that sunflower silage is equal to corn silage for many purposes. Sunflowers for silage appear to offer a very good substitute for corn silage in districts where corn can not be grown.

EFFECT OF OXIDATION OF SULPHUR IN SOILS ON THE SOLUBILITY OF ROCK PHOSPHATE AND ON NITRIFICATION [1]

By O. M. SHEDD [2]

Chemist, Kentucky Agricultural Experiment Station

INTRODUCTION

The widespread use of different forms of phosphate on a large majority of our soils has been justified by the increased returns. In fact, outside of certain areas of our State, the need of phosphate has been so conclusively proved that seldom is the question ever raised as to whether it is necessary but rather what is the most economical kind to use—the soluble form, such as acid phosphate, or the less soluble form, such as ground rock phosphate.

A large amount of work has been done in the greenhouse and the field in comparative tests of the various forms of soluble and insoluble phosphate fertilizers. The writer does not care to discuss in this connection what the results show regarding the relative merits of the two kinds further than to state that on soils very deficient in this element the use of a soluble form has been generally found to be more profitable for immediate returns.

HISTORICAL REVIEW

In the course of some work by the writer during the early part of 1914 (*12–14*),[3] on the sulphur content of Kentucky soils and the effect of this element and its compounds on plant growth, it was found that comparatively large amounts of added sulphur are easily oxidized to sulphate in the soil. The results also indicated that the sulphuric acid formed would act on rock phosphate when present and convert it into a water-soluble form. The time of contact of the mixture of soil, rock phosphate, and sulphur was about 10 weeks. Since the amounts of material used were small and only one soil was tested, the work was not conclusive. It was the writer's intention to continue it later in order to arrive at more definite conclusions, and for this reason it was not mentioned in the earlier publications.

Marés (*11*), in 1869, mentioned the fact that sulphur is converted to sulphate in soils. It was suggested in 1877 by Charles F. Panknin (*11*) of Charleston, S. C., that sulphur, if mixed with ground bone or ground

[1] Published by permission of the Director of the Kentucky Agricultural Experiment Station.

[2] The writer desires to express his appreciation to Dr. A. M. Peter for helpful suggestions and to the Agronomy Department, especially to Prof. P. E. Karraker, for assistance in starting the earlier experiments.

[3] Reference is made by number (italic) to "Literature cited," pp. 344–345.

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phosphate rock, would be oxidized to sulphuric acid when mixed with soil and render the phosphate soluble.

Lipman, McLean, and Lint (*9, 10*), in 1916, probably first demonstrated the practical value of rendering inert phosphates soluble by the oxidation of sulphur in soils. Since then Lipman and McLean (*7, 8, 11*) have continued their experiments and have shown that mixtures of soil, rock phosphate, and sulphur, both with and without manure, would furnish amounts of soluble phosphate that could be substituted for commercial acid phosphate. Furthermore, pot experiments conducted by them have shown that this product compares very favorably in value with the commercial article.

Brown and coworkers (*4, 5, 6*) obtained some favorable results on sulphofication in Iowa soils and its effect on the availability of phosphates. Ames and Richmond, in Ohio (*1, 2, 3*), have made some interesting experiments of this kind, although part of their work has included other phases of sulphur oxidation. Others might be mentioned, but a fairly complete bibliography of the whole sulphur problem in its relation to soils and plant growth is given by McLean (*11*).

The earlier work demonstrated, as was to be expected, that various types of soils have different sulphofying powers and consequently different capacities to render phosphate soluble. For this reason Dr. Lipman, of the New Jersey Agricultural Experiment Station, suggested that it would be desirable to carry on compost experiments with sulphur, rock phosphate, and soils from various localities. Only in this way would it be possible to find whether or not the process would prove of general application.

With this idea in view, and following his suggestion as to the amounts of material to use, the first compost experiments were started here in May, 1917. In the meantime, however, the war had come on; and the increased cost of acid phosphate, due to the acute shortage of sulphuric acid required in the manufacture of munitions, brought this subject to the attention of the National Research Council, Council of National Defense.

Since it appeared at the time that the supply of sulphuric acid allowed to the fertilizer industry would have to be greatly curtailed, if not entirely stopped, this process would, if proved satisfactory, have offered a desirable substitute for commercial acid phosphate. For this reason, in November, 1917, the work was taken in charge by the Agricultural Committee of the National Research Council mentioned and carried on as a war emergency measure. The cooperators in the work were designated as experts in the above council and asked to continue the work as outlined by their committee. As a result, part of the experiments described here were carried on according to the directions sent out by this committee, and others which will be described later were carried on at the same time.

EXPERIMENTAL WORK

EXPERIMENTS WITH COMPOSTS OF ROCK PHOSPHATE, SULPHUR, SOIL, AND MANURE

The materials used were as follows: The rock phosphate was Tennessee brown rock containing 14.2 per cent of phosphorus and guaranteed 95 per cent to pass a 100 mesh sieve. The sulphur was ground brimstone or crude sulphur. The soil was a fertile bluegrass soil obtained from the Station farm and had a high phosphorus content. The manure was partly rotted horse manure, fairly free from straw. The sulphofying soil or starter was obtained from Dr. H. C. McLean, of the New Jersey Agricultural Experiment Station. This starter was simply a mixture of soil, rock phosphate, and sulphur, in which the sulphofying organisms were numerous and active.

Composts No. 1 to 4 were prepared on May 4, 1917, as follows:

TABLE I.—*Composition of composts 1 to 4 (in pounds)*

Ingredients.	No. 1.	No. 2.	No. 3.	No. 4.
Soil	475	375	425	325
Manure	25	25	25	25
Rock phosphate	.....	100	.....	100
Sulphur	.....	.....	50	50
Total	500	500	500	500

The ingredients in each compost were thoroughly mixed, but no starter was added. On November 20, 1917, however, ¾ pound of starter was thoroughly mixed with each compost heap.

The determinations include acidity, water-soluble phosphorus, ammonium-citrate-soluble phosphorus, total phosphorus, sulphates, nitrites, nitrates, total nitrogen, and moisture.

The methods used for acidity, sulphates, and water-soluble and ammonium-citrate-soluble phosphorus were practically the same as those given by Lipman, McLean, and Lint (*10*), with the following exceptions: In the water-soluble phosphorus determinations, 20 gm. of compost were used and phosphorus was determined at first on 16 gm. and later on smaller aliquots. In the ammonium-citrate-soluble phosphorus determinations, 20 gm. of compost were digested with 100 cc. of ammonium citrate solution, and 2-gm. aliquots were used. In the last two determinations, however, in all cases the amount was reduced to 2 gm. in the digestion and 1-gm. aliquots in the determination.

In all citrate-soluble phosphorus determinations made on and after June 19, 1918, the procedure recommended by Dr. J. W. Ames, of the Ohio Agricultural Experiment Station, was used as follows: The aliquot solutions were evaporated with 5 cc. of 50 per cent magnesium nitrate

solution, ignited, then 10 cc. concentrated nitric acid added, evaporated, and ignited again to destroy organic matter. The residue was taken up with dilute hydrochloric acid, evaporated to dehydrate silica, taken up with hydrochloric acid and water, filtered, and phosphorus determined in filtrate. This process was found to be more satisfactory to destroy organic matter.

TABLE II.—*Relative acidity of water-free composts 1 to 4*

[Expressed in cubic centimeters of *N*/50 sodium hydroxid required to neutralize 100 gm. water-free compost]

Date.	No. 1, soil and manure.	No. 2, soil, manure, and rock phosphate.	No. 3, soil, manure, and sulphur.	No. 4, soil, manure, rock phosphate, and sulphur.
1917.				
May 8	Neutral.	2. 31	3. 52	4. 59
24	...do.....	5. 54	11. 37	22. 77
June 6	1. 97	5. 91	61. 52	38. 88
23	2. 27	12. 92	70. 82	56. 60
July 6	2. 39	21. 59	64. 60	61. 71
21	4. 97	36. 49	83. 28	70. 76
Aug. 3	3. 09	26. 03	73. 48	66. 29
17	2. 96	30. 25	60. 21	48. 91
31	3. 38	34. 84	93. 31	68. 40
Sept. 22	3. 18	46. 90	155. 95	61. 95
Oct. 12	4. 81	61. 87	270. 47	87. 79
Nov. 7	2. 44	48. 28	222. 80	70. 30
Dec. 13	3. 41	46. 86	204. 32	78. 04
1918.				
Jan. 18	4. 11	40. 34	158. 23	78. 95
May 21	5. 85	116. 43	1, 049. 03	569. 58
Aug. 14	3. 61	84. 62	2, 821. 34	1, 925. 83
1919.				
May 6	2. 72	72. 48	1, 464. 57	868. 00

In addition to the determinations recommended, it was thought that it might prove of interest to make a study of the nitrites, nitrates, and total nitrogen in these experiments. Since nitrification has generally been found to proceed more favorably in a neutral or slightly alkaline medium, this afforded an opportunity to follow its course in an acid one as well as in the presence of phosphate. Determinations of the total nitrogen were made at the beginning and end of the experiment to find out the effect which composting under these conditions would have on the nitrogen content.

The magnesium nitrate method for total phosphorus, the Griess-Ilosvay method for nitrites, and the Kjeldahl method for total nitrogen, modified to include nitrates, were used for these various determinations.

The nitrates were determined as follows: To the equivalent of 50 gm. of water-free compost were added 125 cc. distilled water, taking into account the water content of the compost. To this was added a small

amount of calcium carbonate, C. P., the whole shaken every five minutes for half an hour, filtered, and the nitrate determined in an aliquot by the phenol-disulphonic acid method by means of a Duboscq colorimeter.

The composts were at first kept in a barn and covered with burlap sacks to prevent rapid evaporation. They were maintained at 15 to 20 per cent water content throughout the experiment. Tap water was used until November 20, 1917, and distilled water after that date. At this time the piles were moved to the heated part of the greenhouse. They were stirred every 10 to 14 days until October 17, 1918, at which time ½-gallon samples were removed to the laboratory and kept loosely covered but stirred only once afterwards. All determinations were made in duplicate.

The averages for the various determinations are given in Tables II to V.

TABLE III.—*Percentage of water-soluble and ammonium-citrate-soluble phosphorus in water-free composts 1 to 4*

Date.	No. 1, soil and manure.		No. 2, soil, manure, and rock phosphate.		No. 3, soil, manure, and sulphur.		No. 4, Soil, manure, rock phosphate, and sulphur.	
	Water-soluble phosphorus.	Ammonium-citrate-soluble phosphorus.	Water-soluble phosphorus.	Ammonium-citrate-soluble phosphorus.	Water-soluble phosphorus.	Ammonium-citrate-soluble phosphorus.	Water-soluble phosphorus.	Ammonium-citrate-soluble phosphorus.
1917.								
May 12	0.003	0.022	0.006	0.027	0.006	0.017	0.007	0.047
22	.005	.012	.008	.024	.006	.012	.008	.016
June 4	.003	.012	.005	.017	.007	.012	.008	.020
18	.006	.016	.009	.058	.006	.064	.009	.089
July 2	.005	.012	.010	.060	.006	.023	.009	.054
16	.005	.035	.009	.079	.003	.069	.007	.072
30	.007	.055	.011	.072	.005	.067	.011	.080
Aug. 13	.006	.027	.009	.060	.006	.048	.011	.065
27	.005	.016	.009	.046	.007	.051	.013	.063
Sept. 17	.007	.050	.011	.085	.014	.096	.010	.093
Oct. 8	.006	.040	.010	.060	.024	.121	.009	.082
Nov. 5	.009	.032	.011	.058	.024	.027	.013	.044
Dec. 10	.011	.088	.013	.138	.023	.176	.014	.144
1918.								
Jan. 15	.008	.075	.012	.118	.012	.160	.011	.134
May 15	.011	.070	.012	.128	.084	.297	.146	.330
Aug. 8	.010	.107	.008	.163	.014	.546	.394	2.007
Oct. 10	.....	.084	.....	.160	.....	.515	.....	2.451
1919.								
Jan. 22	.....	.063	.....	.145	.....	.552	.....	2.280
Apr. 14	.....	.....	.....	.222.	.....	.....	.....	2.822
24	.005	.110	.007	.....	.110	.633	.564	.....
Percentage of total phosphorus	.627	.627	3.176	3.176	.767	.767	3.329	3.329
Maximum percentage of total phosphorus made soluble	1.3	14.0	0.2	6.1	13.6	80.3	16.8	83.6

TABLE IV.—*Percentage of sulphate sulphur in water-free composts 1 to 4*

Date.	No. 1, soil and manure.	No. 2, soil, manure, and rock phosphate.	No. 3, soil, manure, and sulphur.	No. 4, soil, manure, rock phosphate, and sulphur.
1917.				
May 8	0.007	0.064	0.013	0.073
Oct. 15	.056	.369	.580	.436
1918.				
May 21	.087	.508	1.137	1.353
Aug. 14	.093	.561	1.971	3.059
1919.				
May 6	.091	.617	2.714	5.607
Percentage of added sulphur converted to sulphate			27.01	55.34

TABLE V.—*Parts per million of nitrite, nitrate, and total nitrogen in water-free composts 1 to 4*

Date.	No. 1, soil and manure.		No. 2, soil, manure, and rock phosphate.		No. 3, soil, manure, and sulphur.		No. 4, soil, manure, rock phosphate, and sulphur.	
	Nitrite nitrogen.	Nitrate nitrogen.	Nitrite nitrogen.	Nitrate nitrogen.	Nitrite nitrogen.	Nitrate nitrogen.	Nitrite nitrogen.	Nitrate nitrogen.
1917.								
May 9	1.40	48	0.95	25	1.40	13	0.73	27
23	2.58	59	2.33	35	3.10	62	1.23	94
June 5	.60	104	.80	72	.07	94	.31	114
19	.08	200	.10	112	.02	153	.03	174
July 3	.16	155	.19	103	.09	175	.15	190
17	.18	245	.25	128	.19	198	.26	285
31	.20	255	.44	148	.15	163	.19	258
Aug. 14	.16	345	.42	265	.14	240	.16	293
28	.13	303	.25	173	.12	228	.15	273
Sept. 20	.07	383	.10	260	.05	423	.08	410
Oct. 9	.06	323	.15	238	.04	323	.06	330
Nov. 6	.11	310	.29	258	.04	298	.15	310
Dec. 12	.13	513	.36	390	.04	440	.15	443
1918.								
Jan. 16	.21	393	.29	290	.11	370	.23	353
May 15	.16	503	.35	323	.02	353	.02	308
Aug. 10	.04	498	.14	280	None	335	None	83
Parts per million total nitrogen in water-free composts, May 11, 1917	2,609	2,609	2,145	2,145	2,424	2,424	2,059	2,059
Parts per million total nitrogen in water-free compost, May, 1919	a_3, 145 b_2, 443 c_2, 005	a_3, 145 b_2, 443 c_2, 005	a_2, 749 b_2, 020 c_1, 801	a_2, 749 b_2, 020 c_1, 801	a_3, 083 b_2, 023 c_1, 967	a_3, 083 b_2, 023 c_1, 967	a_2, 408 b_1, 705 c_1, 712	a_2, 408 b_1, 705 c_1, 712
Percentage of gain in total nitrogen	20.5	20.5	28.2	28.2	27.2	27.2	16.9	16.9
Maximum parts per million nitrate nitrogen found	513	513	390	390	440	440	443	443
Maximum percentage of original total nitrogen nitrified	17.8	17.8	17.0	17.0	17.6	17.6	20.2	20.2

[a] Composts had been kept in laboratory since Oct. 17, 1918, in Mason jars with tops on but air not excluded.
[b] Moist composts had been kept in Mason jars with air excluded since Oct. 10, 1918.
[c] Composts had been outside exposed to weather since Oct. 17, 1918.

EXPERIMENTS WITH COMPOSTS OF ROCK PHOSPHATE, SULPHUR, AND SOIL

Composts 5 to 10 were prepared on December 20, 1917, from the same materials as were used in the experiments just described. The formula is given in Table VI.

TABLE VI.—*Composition of composts 5 to 10 (in pounds)*

Ingredients.	No. 5, 7, and 9.	No. 6, 8, and 10.
Soil	66⅔	33⅓
Rock phosphates	100	100
Sulphur	.....	33⅓
Starter	⅓	⅓
Total	167	167

The proportions of the various ingredients were the same as those recommended by the Agricultural Committee of the National Research Council for cooperative study, except that the amounts were reduced for these experiments. Otherwise No. 5 and 6 were carried on according to the Committee's directions.

The starter was first mixed with 10 pounds of soil, the whole moistened and stirred every day for 3 days, then added to the remainder of the soil. The rock phosphate and sulphur were mixed separately, and finally all were thoroughly mixed together. These composts were maintained at 15 to 20 per cent moisture content with distilled water and were stirred every 7 to 10 days. All except No. 9 and 10 were kept in the heated part of the greenhouse during the winter.

Since the writer thought it might prove of some interest to study the behavior of these composts under different conditions, No. 7 and 8 were prepared at the same time and kept at the same temperature and moisture content but not stirred throughout the experiments. Composts in a duplicate set, No. 9 and 10, were treated in the same manner as No. 5 and 6, except that they were kept in the unheated part of the greenhouse during the winter. During the first four months No. 9 and 10, while kept at a temperature somewhat above that on the outside, were subjected to a very much lower temperature than the remainder. Especially was this true during the winter, which was the coldest on record. Since May, 1918, however, all have been at the same temperature.

The same determinations were made and the same methods used as in the other experiments except that the total nitrogen was not estimated. The results obtained are given in Tables VII to X.

TABLE VII.—*Relative acidity of water-free composts 5 to 10*

[Expressed in cubic centimeters of *N*/50 sodium hydroxid required to neutralize 100 gm. water-free compost]

Date.	Heated.				Cold, stirred.	
	Stirred.		Not stirred.			
	No. 5, soil and rock phosphate.	No. 6, soil, rock phosphate, and sulphur.	No. 7, soil and rock phosphate.	No. 8, soil, rock phosphate, and sulphur.	No. 9, soil and rock phosphate.	No. 10, soil, rock phosphate, and sulphur.
1917.						
Dec. 27	1.80	2.00	1.80	2.00	1.80	2.00
1918.						
Jan. 22	2.40	13.80	.....	.....	2.00	2.60
Feb. 28	4.00	52.00	4.00	13.60	.....	.....
Mar. 27	4.60	108.60	.....	.....	3.00	64.60
May 3	15.00	175.60	5.00	81.00	.....	.....
June 24	13.60	385.60	.....	.....	10.60	225.60
Aug. 24	11.60	1,363.00	4.60	365.00	7.60	760.60
1919.						
May 6	8.50	906.02	4.99	191.36	5.73	1,151.26

TABLE VIII.—*Percentage of water-soluble and ammonium-citrate-soluble phosphorus in water-free composts 5 to 10*

Date.	Heated.								Cold, stirred.			
	Stirred.				Not stirred.							
	No. 5, soil and rock phosphate.		No. 6, soil, rock phosphate, and sulphur.		No. 7, soil and rock phosphate.		No. 8, soil, rock phosphate, and sulphur.		No. 9, soil and rock phosphate.		No. 10, soil, rock phosphate, and sulphur.	
	Water-soluble.	Ammonium-citrate-soluble.	Water-soluble.	Ammonium-citrate-soluble.	Water-soluble.	Ammonium-citrate-soluble.	Water-soluble.	Ammonium-citrate-soluble.	Water-soluble.	Ammonium-citrate-soluble.	Water-soluble.	Ammonium-citrate-soluble.
1917.												
Dec. 22	0.014	0.063	0.026	0.063	0.014	0.063	0.026	0.063	0.014	0.063	0.026	0.063
1918.												
Jan. 22	.012	.104	.036	.115	.....	.....	.....	.....	.012	.114	.023	.113
Feb. 25	.012	.108	.009	.126	.011	.123	.011	.122	.....	.....	.....	.....
Mar. 26	.008	.112	.015	.151	.....	.....	.....	.....	.010	.120	.014	.142
Apr. 30	.012	.094	.040	.174	.017	.119	.022	.139	.....	.....	.....	.....
June 19	.018	.121	.118	.378	.....	.....	.....	.....	.020	.124	.065	.262
Aug. 21	.010	.123	.425	1.054	.012	.133	.071	.356	.....	.....	.....	.....
26	.....	.....	.....	.....	.....	.....	.....	.....	.014	.140	.294	.733
Oct. 11	.....	.141	.....	1.183	.....	.114	.....	.416	.....	.166	.....	.975
1919.												
Jan. 24	.....	.126	.....	1.874	.....	.113	.....	.638	.....	.141	.....	1.644
Apr. 15	.....	.337	.....	2.532	.....	.....	.....	1.029	.....	.....	.....	2.582
24	.007	.....	.774	.....	.007	.324	.199	.....	.012	.305	.984	.....
Percentage of total phosphorus	9.576	9.576	9.184	9.184	9.576	9.576	9.184	9.184	9.576	9.576	9.184	9.184
Maximum percentage of total phosphorus made soluble	.04	2.9	8.1	26.9	.03	2.7	1.9	10.5	.06	2.5	10.4	27.4

TABLE IX.—*Percentage of sulphate sulphur in water-free composts 5 to 10*

Date.	Heated.				Cold, stirred.	
	Stirred.		Not stirred.			
	No. 5, soil and rock phosphate.	No. 6, soil, rock phosphate, and sulphur.	No. 7, soil and rock phosphate.	No. 8, soil, rock phosphate, and sulphur.	No. 9, soil and rock phosphate.	No. 10, soil, rock phosphate, and sulphur.
1917.						
Dec. 28	0.258	0.253	0.258	0.253	0.258	0.253
1918.						
Jan. 24	.193	.367	.....	.....	.175	.200
Mar. 7	.225	.422	.177	.304	.....	.....
Apr. 1	.249	.550	.....	.....	.221	.446
May 6	.261	.676	.169	.399	.....	.....
June 25	.276	.937	.....	.....	.226	.751
Aug. 26	.289	1.305	.185	.901	.226	1.230
1919.						
May 6	.350	4.227	.206	1.902	.306	3.682
Percentage of added sulphur converted to sulphates	.....	19.91	.....	8.26	.....	17.19

TABLE X.—*Parts per million of nitrate nitrogen in water-free composts 5 to 10*

Date.	Heated.				Cold, stirred.	
	Stirred.		Not stirred.			
	No. 5, soil and rock phosphate.	No. 6, soil, rock phosphate, and sulphur.	No. 7, soil and rock phosphate.	No. 8, soil, rock phosphate, and sulphur.	No. 9, soil and rock phosphate.	No. 10, soil, rock phosphate, and sulphur.
1917.						
Dec. 24	7	3	7	3	7	3
1918.						
Jan. 23	13	4	.....	.....	6	1
Feb. 27	13	2	5	1	.....	.....
Mar. 27	17	3	.....	.....	1	1
May 1	18	1	9	1	.....	.....
June 21	10	2	.....	.....	13	2
Aug. 23	44	5	10	4	27	3

EXPERIMENTS WITH COMPOSTS OF DIFFERENT AMOUNTS OF SOIL

The experiments described below were carried on to test the effect of the sulphofying soil or starter on mixtures of rock phosphate and sulphur when frequently stirred and kept moistened with distilled water, tap water, and soil extract, respectively, and also when variable amounts

of soil were present. The soil extract was prepared by shaking 200 gm. of soil with 2 liters of distilled water and filtering through paper.

Six sets of composts, No. 11 to 16, were started December 22, 1917. One-half of each set was prepared without sulphur and one-half with sulphur, as shown in Table XI.

TABLE XI.—*Composition of composts 11 to 16 (in grams)*

Ingredients.	Without sulphur.	With sulphur.
Rock phosphate	200	150
Sulphur		50
Starter	4	4
Total	204	204

The first three sets were maintained at 20 per cent moisture content—No. 11 with distilled water, No. 12 with tap water, and No. 13 with soil extract. The last three sets were maintained at 20 per cent moisture content with tap water. Two gm. of Station farm soil (the same kind of soil used in composts 1 to 10) were added to compost No. 14, 10 gm. to No. 15, and 20 gm. to No. 16. All were stirred every 7 to 10 days. The ammonium-citrate-soluble phosphorus was determined on July 22, 1918, after a period of about 30 weeks. The results are given in Table XII.

TABLE XII.—*Percentage of ammonium-citrate-soluble phosphorus in water-free composts 11 to 16, showing gain resulting from presence of sulphur*

Composts with and without sulphur.	No. 11, distilled water added.	No. 12, tap water added.	No. 13, soil extract added.	No. 14, 2 gm. soil added.	No. 15, 10 gm. soil added.	No. 16, 20 gm. soil added.
Compost without sulphur	0.177	0.191	0.157	0.173	0.161	0.157
Compost with sulphur	.245	.272	.346	.296	.323	.350
Gain in compost with sulphur	.068	.081	.189	.123	.162	.193

SULPHOFICATION IN DIFFERENT TYPES OF SOILS

The following experiments were carried on for the purpose of determining the sulphofying powers of different types of soils which were later used in compost experiments. The soils represent the principal types, other than the Trenton, found in Kentucky.

Two 100-gm. portions of air-dry soil were intimately mixed with 0.025 and 0.050 gm. of sulphur, respectively. Sixteen gm. of each were then taken, 100 cc. distilled water was added, and the whole was shaken constantly for 7 hours. The remainder was maintained at 20 per cent moisture content with distilled water and stirred every 7 days. At

the end of 34 days the sulphate was again determined in the same manner. In all experiments the barium sulphate was treated with hydrofluoric acid and reprecipitated from sulphuric acid to purify the precipitate. The results are given in Table XIII.

TABLE XIII.—*Percentage of sulphate sulphur in air-dry Kentucky soils*

County.	Feb. 12, 1918.	Mar. 18, 1918.	Grams of sulphur oxidized	Percentage of sulphur oxidized.
Lawrence:				
Soil with 0.025 gm. sulphur added.	0.002	0.025	0.023	92
Soil with 0.050 gm. sulphur added.	.003	.044	.041	82
Warren:				
Soil with 0.025 gm. sulphur added.	.006	.027	.021	84
Soil with 0.050 gm. sulphur added.	.009	.057	.048	96
Mason:				
Soil with 0.025 gm. sulphur added.	.007	.023	.016	64
Soil with 0.050 gm. sulphur added.	.008	.041	.033	66
Muhlenberg:				
Soil with 0.025 gm. sulphur added.	.006	.026	.020	80
Soil with 0.050 gm. sulphur added.	.006	.046	.040	92
Barren:				
Soil with 0.025 gm. sulphur added.	.004	.020	.016	64
Soil with 0.050 gm. sulphur added.	.004	.042	.038	76
McCracken:				
Soil with 0.025 gm. sulphur added.	.005	.025	.020	80
Soil with 0.050 gm. sulphur added.	.005	.051	.046	92
Madison:				
Soil with 0.025 gm. sulphur added.	.003	.025	.022	88
Soil with 0.050 gm. sulphur added.	.003	.046	.043	86
Jefferson:				
Soil with 0.025 gm. sulphur added.	.002	.020	.018	72
Soil with 0.050 gm. sulphur added.	.003	.043	.040	80

EXPERIMENTS WITH COMPOSTS OF DIFFERENT TYPES OF SOILS

The same rock phosphate and sulphur were used as in the former experiments. The sulphofying soil was from a new lot obtained from the same place as the other. The composts were prepared on March 15, 1917, as follows:

TABLE XIV.—*Composition of composts (in grams) prepared with various Kentucky soils*

Ingredients.	Without sulphur.	With sulphur.
Soil	133⅓	66⅔
Rock phosphate	200	200
Sulphur	.....	66⅔
Starter	6⅔	6⅔
Total	340	340

The same soils were used here as in the sulphofication experiments, and the soil used in the former experiments was also included. The mixtures were kept at 20 per cent moisture content with distilled water in Mason jars covered with watch glasses. They were stirred every 7 to 10 days until September 27, 1918, and after this date they were not stirred but kept moistened. The results are shown in Table XV.

TABLE XV.—*Percentage of ammonium-citrate-soluble phosphorus in water-free composts prepared with various Kentucky soils*

County.	Soil and rock phosphate.		Soil, rock phosphate, and sulphur.	
	Sept. 27, 1918.	Mar. 7, 1919.	Sept. 27, 1918.	Mar. 7, 1919.
Lawrence	0.124	0.103	0.456	0.473
Warren	.141	.117	.469	.488
Mason	.132	.122	.411	.523
Muhlenberg	.169	.139	.480	.531
Barren	.135	.126	.436	.470
McCracken	.135	.107	.452	.501
Madison	.130	.125	.517	.549
Jefferson	.162	.136	.493	.501
Fayette	.178	.175	.604	.611

DISCUSSION OF RESULTS

It will be observed in Table III of the earlier experiments that 4 per cent of the phosphorus present in No. 4 was citrate-soluble after 7 months, 60 per cent after 15 months, and 83 per cent after 24 months. The amount in soluble form after 7 months, however, is much less than Dr. Lipman found in his experiments. In fact, in the present experiments no pronounced action developed until the starter was added.

Another interesting fact is that about the same percentages of the total phosphorus present was in water-soluble form in No. 3 and 4; and the same held true for the citrate-soluble form, notwithstanding there was over 400 per cent more phosphorus present in No. 4. The amount found soluble in water, however, was much less than in ammonium citrate in all cases. One reason why larger amounts of soluble phosphorus were not obtained was probably the absorptive capacity of the soil for compounds of this element, which prevented the portion thus occluded from being estimated, although it existed in a soluble form.

It will be observed further that about 13 per cent of the phosphate naturally present in this soil has been converted into a water-soluble form and 80 per cent into citrate-soluble form. Composting without sulphur has also shown some benefit, since 14 per cent of the phosphorus in No. 1 and 6 per cent in No. 2 was citrate-soluble, although the latter contained over 500 per cent more total phosphorus than No. 1. The apparently inconsistent amounts of total phosphorus shown in the earlier compost experiments are explained by the fact that a large amount of soil was used in preparing these experiments, and since the quantity of phosphate was large and not evenly distributed, probably a uniform mixture was not obtained. Subsequent mixings of the separate piles, however, during the experiments and before the totals were determined make the analyses for this element trustworthy.

It will further be observed in Table V that nitrification took place in all piles regardless of the amount of acid formed. There was an irregular but gradual increase in nitrates until December 12, 1917, at which time the maximum amounts were found in all cases. These maximums were approximately 17 per cent of the original total nitrogen present in each except No. 4, in which there was 20 per cent. The actual amounts of nitrogen nitrified at this time were 232 mgm. in No. 1, 182 mgm. in No. 2, 213 mgm. in No. 3, and 208 mgm. in No. 4. This is of interest when Table II shows that No. 3 contained 60 times more acid at this time. It should be borne in mind that just previous to this the starter had been added and this was the first time that any pronounced activity of the sulphofying organism was apparent, as indicated in the phosphate solubility in Table III.

As the acidity developed there was a distinct loss of nitrate in only one sample, No. 4, which in August, 1918, showed a decrease but still contained 300 per cent more than at the beginning, while the acidity had increased 40,000 per cent.

The results on total nitrogen are not conclusive in regard to the behavior of this element during the experiments. One set of analyses in Table V indicates there had been a gain. The loss in weight due to the decomposition of the manure does not account for this gain. Neither does the nitrogen contained in the amount of tap water added explain it.

On the other hand, there evidently has been a gain in weight of composts No. 3 and 4 on account of sulphofication, and we would expect some loss in nitrogen from the manure during decomposition.

Unfortunately these samples had previously been kept in the laboratory in Mason jars with the tops on loosely so that air was not exlcuded, and they may have taken up ammonia fumes. But if such is the explanation it would seem that the acid composts No. 3 and 4 would have absorbed more than the others. The respective gains in No. 1, 2, 3, and 4 were 0.0536, 0.0604, 0.0659, and 0.0349 per cent.

On the other hand, the composts from which the air was excluded as well as those exposed to the weather showed losses of nitrogen; but these losses may have been caused by denitrification in the former and leaching in the latter. The fact that leaching apparently did not cause a loss of nitrogen in No. 3 and 4 greater than that caused by the exclusion of air indicates that denitrification took place in the samples from which the air was excluded.

In the later experiments, after 17 months' time, about 27 per cent of the phosphorus present had been made citrate-soluble, as shown in Table VIII. In the earlier experiments, in about the same time, 72 per cent was citrate-soluble. After about 8 months, however, nearly 11 per cent of the total phosphorus was citrate-soluble in these experiments as compared with 4 per cent in the earlier ones. This was probably due to better conditions for controlling temperature, although part of the initial good effect was undoubtedly due to the starter.

The results show that the reaction proceeds more rapidly at a high temperature and when the composts are stirred frequently. While No. 6 and 10 at the end showed about the same amounts of citrate-soluble phosphorus, the effect of temperature is illustrated during the first months of the experiments. In fact, it required the heat of the summer months before pronounced activity was shown in any experiment.

Because much less soil and no manure was used in the latter work, the nitrate figures are not so significant as in the other experiments. Nevertheless, as much nitrate was present at higher acid concentration as was found at the beginning, or more.

That the presence of the sulphofying organism in the amount of starter used is not sufficient to continue the reaction as rapidly as does the further addition of soil bacteria can be seen in Table XII. Another fact to be considered in this connection is that increasing the amounts of soil slightly favored the reaction, although soil water obtained from a large mass of the same soil gave about as good results.

The sulphofying power of different types of soil varied somewhat, but all had the capacity to oxidize sulphur. The relative amounts of sulphur oxidized in individual cases did not vary materially, although the totals, while small, differed as much as 100 per cent. None of these soils,

however, when tried in compost experiments equaled the soil used in the large experiments in its capacity to render phosphate soluble.

That the solubility of the phosphate has been caused by the oxidation of the sulphur is demonstrated by the parallel rise in acidity and sulphates found.

Computing from November, 1917, when the starter was added in the earlier work and when for the first time active sulphofication was shown, the duration of the earlier experiments as well as of the latter was 17 months. On the basis of 100 pounds water-free composts, it will be seen that about 2¾ pounds of phosphorus were made citrate-soluble in No. 4 and 2½ pounds in No. 6 and 10. The advantage in favor of No. 4 may be due to either the manure or the larger mass of soil and probably to both. Taking into account the cost of materials, it appears that No. 4 is the more desirable compost.

It is the writer's opinion that the presence of some manure and more soil would have been advantageous in the later work because it probably would have provided a means of increased bacterial action as well as assisted in maintaining the moisture content.

It would appear from the work described here that while this procedure would furnish a means for the manufacture of acid phosphate in an emergency such as confronted us at the time, under ordinary conditions the average consumer would object to the time and labor involved. Aside from this, however, it is of scientific interest; and as better methods of inoculation are developed the process may be so simplified that it may become of immediate practical benefit.

SUMMARY

(1) Compost experiments of rock phosphate, sulphur, soil, and manure show, after 24 months' time, that about 17 and 84 per cent of the total phosphorus had been converted into a water-soluble and ammonium-citrate-soluble form, respectively. Whether the amounts were really larger than the above could not be determined because of the limitations of the method.

(2) Sulphofication did not proceed as rapidly as when an inoculation was made with the sulphofying organism, and when this was done the time of the sulphofication may be considered to be reduced nearly one-third.

(3) Composting under the same conditions but omitting the sulphur also showed favorable results in rendering the soil phosphate or that added in rock sulphate soluble, but not to the same extent as when sulphur was present.

(4) Nitrification was found to proceed to a certain extent regardless of the acid formed by the sulphur oxidation. The amounts of nitrogen

found to be nitrified amounted to approximately 20 per cent of the total originally present.

(5) The changes that took place in the nitrogen content of the composts are interesting and seem to indicate that there may have been some fixation of this element from the air, although the results are not conclusive and need verification.

(6) Sulphofication was found to take place in all the soils examined but varied somewhat according to the type. When 25 and 50 mgm. of sulphur were added to 100 gm. of soil, about the same percentage of the total was oxidized in a given time.

(7) Inoculation of mixtures of rock phosphate and sulphur was not sufficient to promote rapid sulphofication. It required in addition soil or soil water.

(8) None of the soils tested equaled the Fayette County sample in its capacity to render phosphate soluble when composted with rock phosphate and sulphur.

(9) That the production of soluble phosphate was caused by the presence of sulphuric acid generated by the oxidation of the sulphur is demonstrated by the parallel rise in acidity and sulphate.

(10) The best conditions to promote the reaction are initial inoculation, high temperature, thorough aeration, and a fair moisture content. Other contributing factors are the proportions of the different ingredients and probably their mass.

(11) Taking into account the cost of materials, the compost containing the larger amount of soil and some manure proved more desirable.

(12) The acid phosphate made by this procedure has just as good a physical condition as the commercial product and would be cheaper if the time and labor involved in its manufacture are disregarded. However, these factors would be the chief causes of objection offered by the consumer. With further work on the composition of the mixtures and methods of inoculation it is possible that the process may be simplified so that it may prove of immediate practical application.

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EFFECT OF LIME UPON THE SODIUM-CHLORID TOLERANCE OF WHEAT SEEDLINGS

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INTRODUCTION

The work reported here is a continuation of that previously reported in Bulletin 149 of the Bureau of Chemistry. In this former work the beneficial effect of lime upon soils which have become acid by the continued use of potassium chlorid or potassium sulphate was pointed out. The present work takes up an equally important rôle of lime—its effect upon some of the salts commonly occurring in "alkali" soils.

The concentration of nutrient salts best suited to plant growth [2] is entirely different in sand and in solution cultures. Seedlings grown in a solution of 2,500 parts per million of nutrient salts suffer, while those grown in sand and watered continuously with the solution, with free drainage, produce vigorous plants. From this it appears that inert material, such as sand, clay, and soil, might materially affect the toxic limit of dissolved salts. It was, therefore, desired to see whether a plant growing in a stiff soil would be more resistant to sodium chlorid, which is one of the salts found in alkali soils, than one growing in a sandy soil or in a solution.

EXPERIMENTS

SERIES 1

Several small glass jars having a capacity of 250 cc. were filled with sodium-chlorid solutions of the following concentrations:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	Distilled water.
2	500 parts per million of sodium chlorid.
3	1,000 parts per million of sodium chlorid.
4	2,000 parts per million of sodium chlorid.
5	3,000 parts per million of sodium chlorid.
6	4,000 parts per million of sodium chlorid.

[1] Much appreciation on the part of the authors is due to Dr. J. Davidson for his painstaking review and correction of the manuscript.

[2] BREAZEALE, J. F. EFFECT OF THE CONCENTRATION OF THE NUTRIENT SOLUTION UPON WHEAT CULTURES. *In* Science, v. 22, no. 553, p. 146–149. 1905.

Six wheat seedlings were introduced into these jars and held in place through slits in the corks by rubber bands. The seedlings had been sprouted in pans of distilled water on floating perforated aluminum disks and allowed to grow for three days, or until they were about 10 cm. long, before being placed in the solutions. At the same time a similar set of jars, with small holes drilled in the bottom for drainage, were filled with quartz sand and planted with wheat seed. These sand cultures were watered almost continuously during the day with sodium-chlorid solution of the same strength as that used in the corresponding water culture. By forcing a large amount of solution through each jar the concentration of the solution in the sand was kept fairly constant. This was frequently checked by titrating the solution before and after it passed through the sand.

When the plants were 8 days old they were removed and photographed. The plants grown in solution are shown in Plate 38, A, and those grown in sand in Plate 38, B. It will be seen from Plate 38, A, that the toxic action of sodium chlorid manifests itself, especially upon the root development, in all the concentrations tried. This was not true in the sand culture, in which an increase in growth was noticed up to a concentration of 1,000 parts per million. In comparing the two illustrations it will be noticed also that the higher concentrations were more toxic in the sand than in the solution cultures. This was probably due to the fact that sand cultures were started from the seed, while the solution cultures were started from seedlings 3 days old, the plant being apparently more sensitive to sodium chlorid during the first few days of its growth.

This necessitated a change in the method of sprouting the seedlings. In all the experiments hereafter to be described the seedlings were sprouted in the same concentration of the solution as that in which they were afterwards to be placed.

SERIES 2

The next experiment was begun on February 26, with the following solutions:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	Distilled water.
2	500 parts per million of sodium chlorid.
3	1,000 parts per million of sodium chlorid.
4	1,500 parts per million of sodium chlorid.
5	2,000 parts per million of sodium chlorid.
6	3,000 parts per million of sodium chlorid.
7	4,000 parts per million of sodium chlorid.

Large enameled pans holding about 3 liters were filled with the solutions of the different concentrations, and perforated aluminum disks were floated in them. Wheat seeds were sprouted upon the disks.

As soon as the radicles appeared, usually at the end of 24 hours, the seeds for the sand or soil cultures were taken out and planted. The pans were allowed to stand until the shoots of the other seeds had reached a height of about 1 cm., when they were transferred to the culture bottles. In addition to the sand and solution cultures, this experiment included a series of bottles filled with very fine soil—a volcanic ash obtained from Jamaica, consisting of about 98 per cent of iron and aluminum oxid, principally iron. The bottles of soil and sand were watered with these solutions before planting until the drainage titrated the same for chlorid as did the original solutions. This took about 2 days in the soil cultures, since only about 250 cc. of the solution moved through the clay in an hour. With the sand it took a much shorter time.

On March 5, when the plants were 8 days old, they were photographed (Pl. 39).

As in Plate 38 it will be seen that the toxic action of the sodium chlorid was first noticeable in the solution cultures, even in the 500 parts per million solution, the lowest concentration; and the effect was more and more appreciable, especially upon the root development, as the concentration increased. It was not noticeable in the sand cultures in the lower concentrations, in which the root development showed no marked difference until a concentration of 2,000 parts per million was reached. With the soil cultures, no toxic effect of the sodium chlorid on the growth of the plants could be seen, even in the highest concentration. In fact, the plants growing in the soil and watered almost continuously with a 4,000 parts per million sodium-chlorid solution grew as well as, if not better than, those growing in the same soil and watered with distilled water. The only noticeable effect of the sodium chlorid upon the soil cultures was the tendency to cause lodging, especially as the plants grew older, this tendency increasing with the amount of sodium chlorid. This is shown in Plate 40, A, which shows the plants when 9 days old.

SERIES 3

On March 12 another set of solution, sand, and soil pots was started, with concentrations of sodium chlorid running from 1,000 to 4,000 parts per million. On March 15 one plant was withdrawn from each group where the highest concentration was used and a photograph was made. The 3-day-old seedlings which had been grown in 4,000 parts per million solution (Pl. 40, B) were so small that they were still dependent upon the seed and had not yet begun to feed upon the solution to any appreciable extent. It was evident that the effect of the soil was manifested upon the young plant at a very early stage in its life history.

SERIES 4

To test the effect of the practically inert material represented by the soil, two pots were started, one in a 4,000 parts per million solution of sodium chlorid and the other in pure carbon black, watered with 4,000 parts per million sodium-chlorid solution. The young plants grown in carbon black watered with 4,000 parts per million sodium chlorid were just as poor as those grown in 4,000 parts per million sodium-chlorid solution, indicating that the effect of the soil in overcoming the toxicity of sodium chlorid was due merely to the presence of inert particles.

SERIES 5

On March 6 a set of soil bottles was prepared, arranged on a filter rack so that the drainage could be easily collected, and watered with the following solutions:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	Distilled water.
2	1,000 parts per million of sodium chlorid.
3	1,500 parts per million of sodium chlorid.
4	2,000 parts per million of sodium chlorid.
5	3,000 parts per million of sodium chlorid.
6	4,000 parts per million of sodium chlorid.

When it was found by titration that the drainage was of the same salt content as the original solution, 200 cc. that had passed through the soil and the sand, respectively, were collected from under each pot. These solutions were put into culture bottles and, together with the sand and soil pots, planted with wheat seedlings in the manner before described. We now had plants growing in sand, in the solution that had passed through the sand, in soil, and in the solution that had passed through the soil. The sand and soil pots were watered frequently during the day with their respective solutions until the seedlings were 7 days old, when they were photographed (Pl. 41).

The plants grown in the sand pots differed very little from those grown in the solutions that had passed through the sand (Pl. 41, A). The limit of tolerance for each was 3,000 parts per million of sodium chlorid. No difference in the plants growing in the soil could be detected, even in the highest concentration (Pl. 41, B). Although none of the plants grown in the solution which had passed through the soil were quite so good as the control, excellent plants were obtained even in the highest concentration—4,000 parts per million, which is considerably above the limit of tolerance of sodium chlorid as shown in sand.

The plants were allowed to grow until March 16, or until they were 10 days old. They were then uprooted or removed from the solution and photographed. The plants growing in the sand are shown in Plate 42, A, and those grown in soil in Plate 42, B. The plants grown in the solutions

which had passed through the sand and likewise in the solutions which had passed through the soil were similar to those grown in the sand and soil. It is evident, therefore, that in passing through the soil the solution was so altered as to overcome almost completely the toxic effect of the sodium chlorid.

SERIES 6

On March 13 another set was started with the following solutions:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	4,000 parts per million of sodium chlorid.
2	6,000 parts per million of sodium chlorid filtered through the soil.
3	8,000 parts per million of sodium chlorid filtered through the soil.
4	4,000 parts per million of sodium chlorid filtered through the soil.
5	4,000 parts per million of sodium chlorid added to distilled water that had previously been filtered through the soil.
6	4,000 parts per million of sodium chlorid + 100 parts per million each of sodium nitrate, potassium chlorid, and sodium phosphate.

On March 20 the plants were photographed (Pl. 43, A). It will be seen that vigorous plants were obtained in solution 3 in a concentration of 8,000 parts per million sodium chlorid, or double the amount necessary to stop the growth in distilled water, by simply filtering the salt solution through the soil. Good plants were also obtained in solution 5 to which the salt had been added after the distilled water had been passed through the soil. In solution 6 the addition of the fertilizer salts produced comparatively little better growth than was noted in the control.

Two hundred cc. of water were then passed through a fresh pot of soil and analyzed. The salt content of the solution showed 31 parts per million of calcium oxid. This suggested the possibility that lime might be the cause of the greater tolerance noticed in all the soil pots.

SERIES 7

To test the effect of lime the following set was started:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	4,000 parts per million of sodium chlorid.
2	4,000 parts per million of sodium chlorid + 30 parts per million of calcium sulphate.
3	4,000 parts per million of sodium chlorid + 30 parts per million of calcium oxid.
4	4,000 parts per million of sodium chlorid + 30 parts per million of magnesium bicarbonate.

On March 29 the plants were photographed (Pl. 43, B). The magnesium salt had little effect, but the calcium sulphate and calcium oxid were about equally effective in overcoming the toxic action of sodium chlorid.

SERIES 8

To determine the relation of some other salts to the sodium-chlorid tolerance the following set was started on March 30:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	Distilled water.
2	4,000 parts per million of sodium chlorid + 30 parts per million of calcium sulphate.
3	4,000 parts per million of sodium chlorid + 30 parts per million of calcium oxid.
4	4,000 parts per million of sodium chlorid + 30 parts per million of magnesium sulphate.
5	4,000 parts per million of sodium chlorid + 30 parts per million of barium chlorid.
6	4,000 parts per million of sodium chlorid + 30 parts per million of potassium chlorid.
7	4,000 parts per million of sodium chlorid + 30 parts per million of sodium nitrate.
8	4,000 parts per million of sodium chlorid + 30 parts per million of sodium phosphate.
9	4,000 parts per million of sodium chlorid + 30 parts per million of ferric chlorid.
10	4,000 parts per million of sodium chlorid + 30 parts per million of potassium alum.

At the end of seven days the plants were photographed (Pl. 44). In this experiment the calcium oxid produced the best plants and calcium sulphate the next best. While the effect of the barium chlorid and magnesium sulphate was not marked, it was evident that they exerted some action upon the sodium chlorid, while the potassium chlorid, sodium nitrate, sodium phosphate, ferric chlorid, and alum produced little or no change.

On March 30 two pots of sand were started. No. 1 was watered with 4,000 parts per million sodium-chlorid solution and the other with the same solution to which had been added 30 parts per million of calcium sulphate. The plants were grown for 16 days and then photographed (Pl. 45, A). The beneficial effect of such a minute amount of lime in sand cultures is clearly shown in this experiment.

SERIES 9

In Plate 45, B, are shown 3-day-old plants grown in the following solutions of sodium sulphate:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	4,000 parts per million of sodium sulphate.
2	4,000 parts per million of sodium sulphate + 30 parts per million of calcium sulphate.
3	4,000 parts per million of sodium sulphate + 30 parts per million of calcium oxid.
4	4,000 parts per million of sodium sulphate + 30 parts per million of magnesium sulphate
5	4,000 parts per million of sodium sulphate + 30 parts per million of barium chlorid.

With sodium sulphate the beneficial effects of the calcium salts were just as pronounced as they had been with sodium chlorid, while no benefit resulted from the use of either the barium or magnesium salt.

SERIES 10

Plate 46, A, shows the 3-day-old plants grown in the following solutions:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	Distilled water.
2	4,000 parts per million of sodium sulphate.
3	4,000 parts per million of sodium sulphate + 30 parts per million of potassium chlorid.
4	4,000 parts per million of sodium sulphate + 30 parts per million of sodium nitrate.
5	4,000 parts per million of sodium sulphate + 30 parts per million of ferric oxid.
6	4,000 parts per million of sodium sulphate + 30 parts per million of aluminum oxid.

The oxid of iron and aluminum were here included for the reason that they have shown a marked effect upon slightly acid cultures in previous work with wheat seedlings,[1] but neither these nor the two fertilizers showed any effect.

SERIES 11

Plate 46, B, shows 11-day-old plants which had been grown in the following solutions of sodium bicarbonate:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	2,500 parts per million of sodium bicarbonate.
2	2,500 parts per million of sodium bicarbonate + 30 parts per million of sodium nitrate.
3	2,500 parts per million of sodium bicarbonate + 30 parts per million of potassium chlorid.
4	2,500 parts per million of sodium bicarbonate + 30 parts per million of magnesium sulphate.
5	2,500 parts per million of sodium bicarbonate + 30 parts per million of calcium oxid.

The beneficial effects of the lime are shown here also, although not in so great a degree as with sodium chlorid or sodium sulphate. The experiment with sodium bicarbonate was repeated several times; but while the tops showed distinct effects of the calcium salts, no such marked difference was noticed in the root development.

[1] BREAZEALE, J. F., and LE CLERC, J. A. THE GROWTH OF WHEAT SEEDLINGS AS AFFECTED BY ACID OR ALKALINE CONDITIONS. U. S. Dept. Agr. Bur. Chem. Bul. 149, 18 p., 8 pl. 1912.

SERIES 12

In the experiments previously conducted, the calcium salts were added at the rate of 30 parts per million. Smaller amounts were now added to determine the minimum amount of lime that could exert an appreciable effect upon plants growing in a toxic salt. Plate 47, A, shows plants grown in the following solutions:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	4,000 parts per million of sodium chlorid.
2	Distilled water.
3	4,000 parts per million of sodium chlorid + 40 parts per million of calcium oxid.
4	4,000 parts per million of sodium chlorid + 30 parts per million of calcium oxid.
5	4,000 parts per million of sodium chlorid + 20 parts per million of calcium oxid.

Forty, 30, and 20 parts per million of lime entirely overcame the depressing action of the sodium chlorid and produced plants equal to those grown in distilled water.

SERIES 13

In Plate 47, B, are shown plants grown in the following solutions:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	4,000 parts per million of sodium chlorid.
2	4,000 parts per million of sodium chlorid + 15 parts per million of calcium oxid.
3	4,000 parts per million of sodium chlorid + 10 parts per million of calcium oxid.
4	4,000 parts per million of sodium chlorid + 2 parts per million of calcium oxid.
5	4,000 parts per million of sodium chlorid + 1 part per million of calcium oxid.

From Plate 47 it will be seen that the size of the plants decreases gradually as the concentration decreases from 40 parts to 1 part per million of lime. Much better plants were grown in the solutions containing 2 parts and 1 part per million, respectively, than in the solution containing no lime. It is evident that the presence of calcium salts, even in most minute quantities, materially affects the tolerance of young wheat seedlings for sodium chlorid.

SERIES 14

No satisfactory explanation has yet been given for the effect of calcium salts on the toxic properties of other salts. W. J. V. Osterhout [1] concluded, on the basis of a series of experiments, that entrance of ions of sodium chlorid into the protoplasm was greatly hindered or prevented by

[1] OSTERHOUT, W. J. V. THE PERMEABILITY OF PROTOPLASM TO IONS AND THE THEORY OF ANTAGONISM. *In* Science, n. s. v. 35, no. 890, p. 112–115. 1912.

the presence of a small amount of calcium chlorid and that barium and strontium exerted a similar action. J. Loeb,[1] in his work on the effect of various salts on fishes, concluded similarly that the antagonism of the calcium salts toward certain toxic salts was due to their effect on the permeability of the cell.

Series 14 was introduced to determine the effect of small amounts of lime on the actual absorption of sodium chlorid as shown by the analysis of the ash.

The following solutions were used:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	Distilled water.
2	4,000 parts per million of sodium chlorid.
3	4,000 parts per million of sodium chlorid + 30 parts per million of calcium oxid.

When the plants were 8 days old, 100 were withdrawn from each pan and the amount of ash and chlorin estimated. Tables I and II give the results of the analyses.

TABLE I.—*Analyses of 100 tops*

Concentration of solution.	Ash.	Chlorin, calculated to sodium chlorid.
	Gm.	*Gm.*
Distilled water	0.0462	Trace.
4,000 p. p. m. of sodium chlorid	.0775	0.0320
4,000 p. p. m. of sodium chlorid + 30 p. p. m. of calcium oxid	.0921	.0334
100 whole seeds, ungerminated	.0710	Trace.

TABLE II.—*Analyses of 100 whole plants*

Concentration of solution.	Dry weight.	Chlorin, calculated to sodium chlorid.
	Gm.	*Gm.*
Distilled water	2.077	Trace.
4,000 p. p. m. of sodium chlorid	1.993	0.0553
4,000 p. p. m. of sodium chlorid + 30 p. p. m. of calcium oxid	2.352	.0595

Upon repeating this experiment with three different sets of plants, similar results were obtained each time, showing that there was just as much chlorin in the plants growing in the solution containing lime as in those grown in the solution containing no lime. Within the limits of our experiments, lime is not effective in preventing absorption of sodium chlorid by the plant.

[1] LOEB, Jacques. ÜBER DIE HEMMUNG DER GIFTWIRKUNG VON NaJ, $NaNO_3$, NaCNS UND ANDEREN NATRIUMSALZEN. *In* Biochem. Ztschr., Bd. 43, Heft 3, p. 181–202. 1912.

This same experiment was twice repeated, using sodium sulphate instead of sodium chlorid, with the results shown in Table III.

TABLE III.—*Analyses of 100 whole plants*

Concentration of solution.	Dry weight.	Ash.	Sodium sulphate.
	Gm.	*Gm.*	*Gm.*
Distilled water	2.146	0.0667	Trace.
4,000 p. p. m. of sodium sulphate	2.460	.0958	0.0219
4,000 p. p. m. of sodium sulphate + 30 p. p. m. of calcium oxid	2.550	.1460	.0521

The results with sodium sulphate are even more striking than those with sodium chlorid. Instead of hindering the absorption of sodium sulphate by the plant, the calcium oxid actually stimulated it. Even when calculated upon the basis of grams of dry weight, there is still a preponderance of sodium sulphate in the plants grown in the presence of the small amount of lime.

CONCLUSIONS

(1) The higher tolerance to alkali salts shown by plants in soil and sand than by those grown in water cultures is not due entirely to the physical effect of the presence of solid particles of different degrees of fineness, but also to certain soluble substances which are sometimes present in very small quantities.

(2) Very small amounts of calcium oxid and calcium sulphate overcame the toxic effects of sodium chlorid and sodium sulphate.

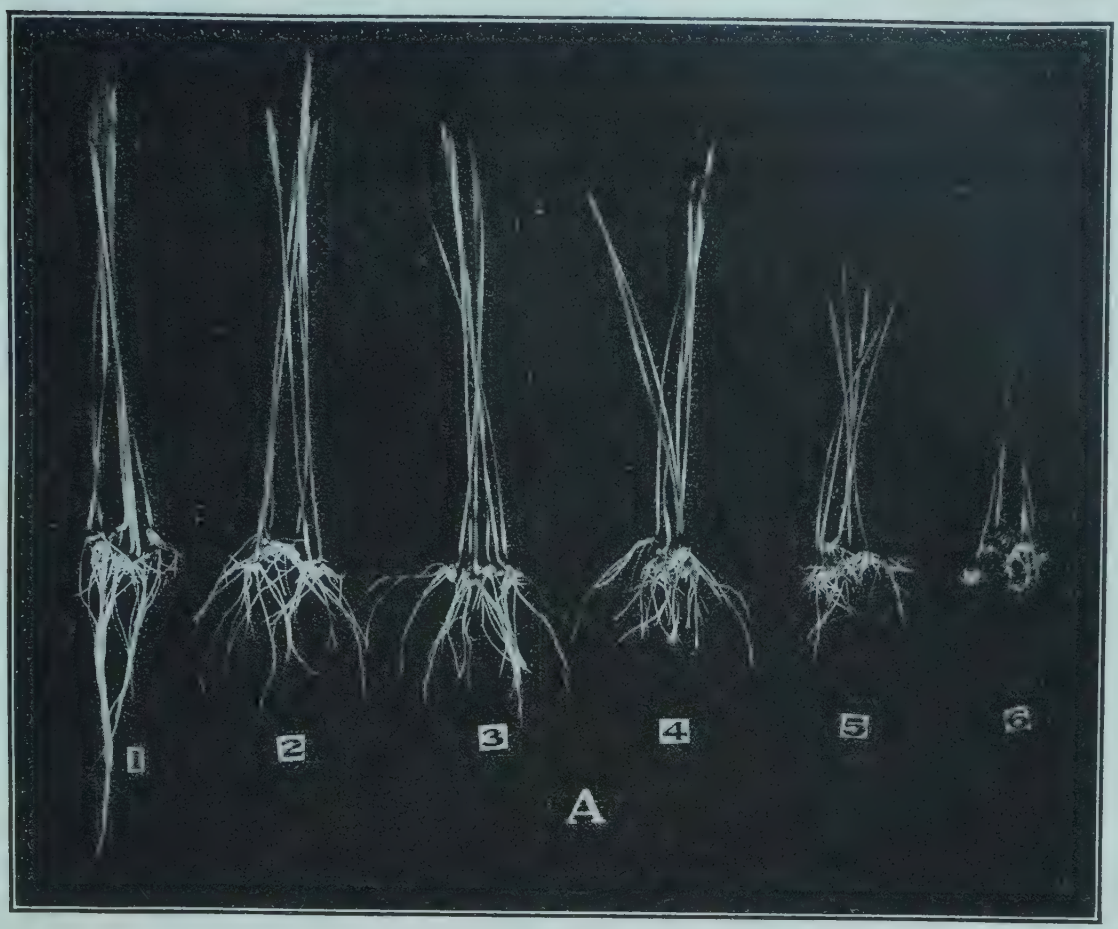
(3) Magnesium sulphate and barium chlorid were slightly antagonistic to sodium chlorid. Potassium chlorid, sodium nitrate, sodium phosphate, ferric chlorid, and alum had no effect on the toxicity of sodium chlorid.

(4) Within the limits of our experiments the presence of lime did not prevent the entrance of sodium chlorid and sodium sulphate into the plant cells. The antagonistic effects of lime would seem to be due not to its effect on the permeability of the cells but to some other cause.

PLATE 38

A.—Seedlings grown in (1) distilled water, (2) 500 parts per million sodium-chlorid solution, (3) 1,000 parts per million sodium-chlorid solution, (4) 2,000 parts per million sodium-chlorid solution, (5) 3,000 parts per million sodium-chlorid solution, and (6) 4,000 parts per million sodium-chlorid solution.

B.—Seedlings grown in sand and watered with (1) distilled water, (2) 500 parts per million sodium-chlorid solution, (3) 1,000 parts per million sodium-chlorid solution, (4) 2,000 parts per million sodium-chlorid solution, (5) 3,000 parts per million sodium-chlorid solution, and (6) 4,000 parts per million sodium-chlorid solution.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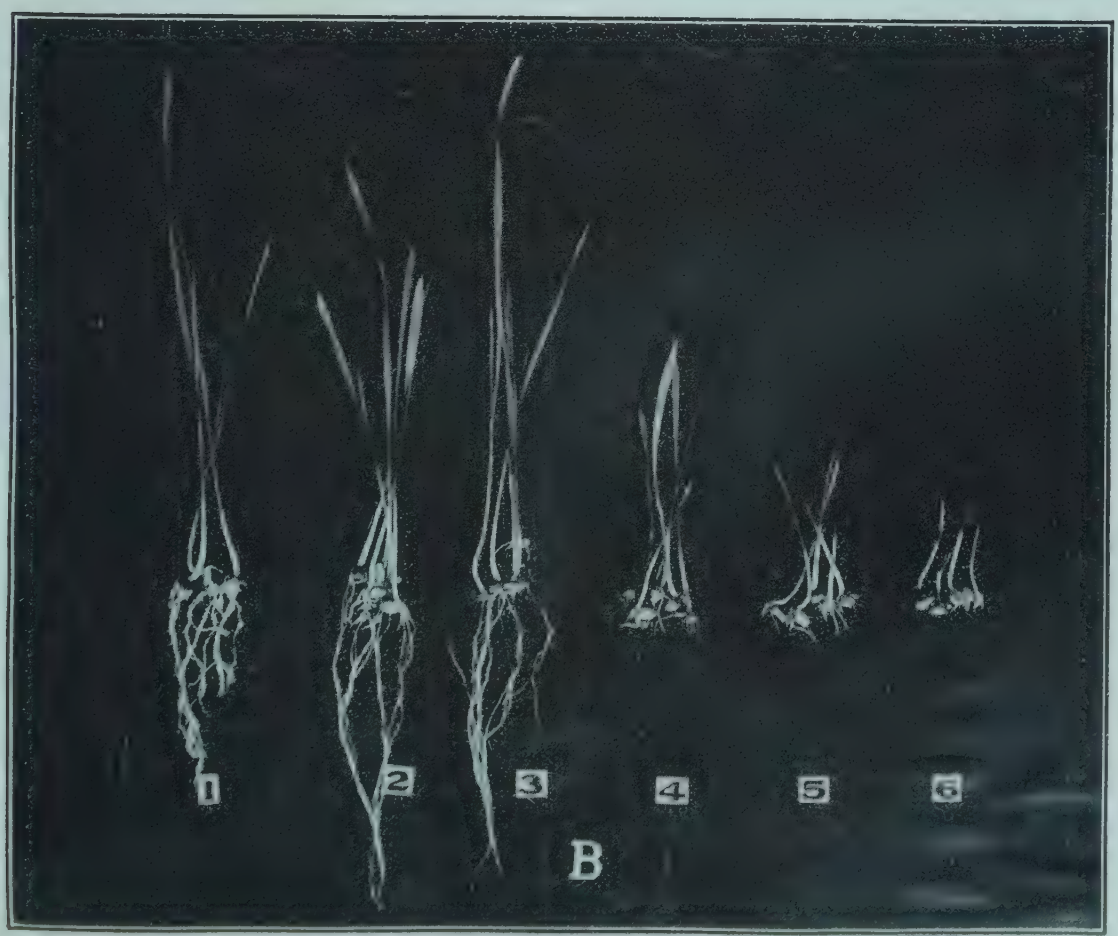
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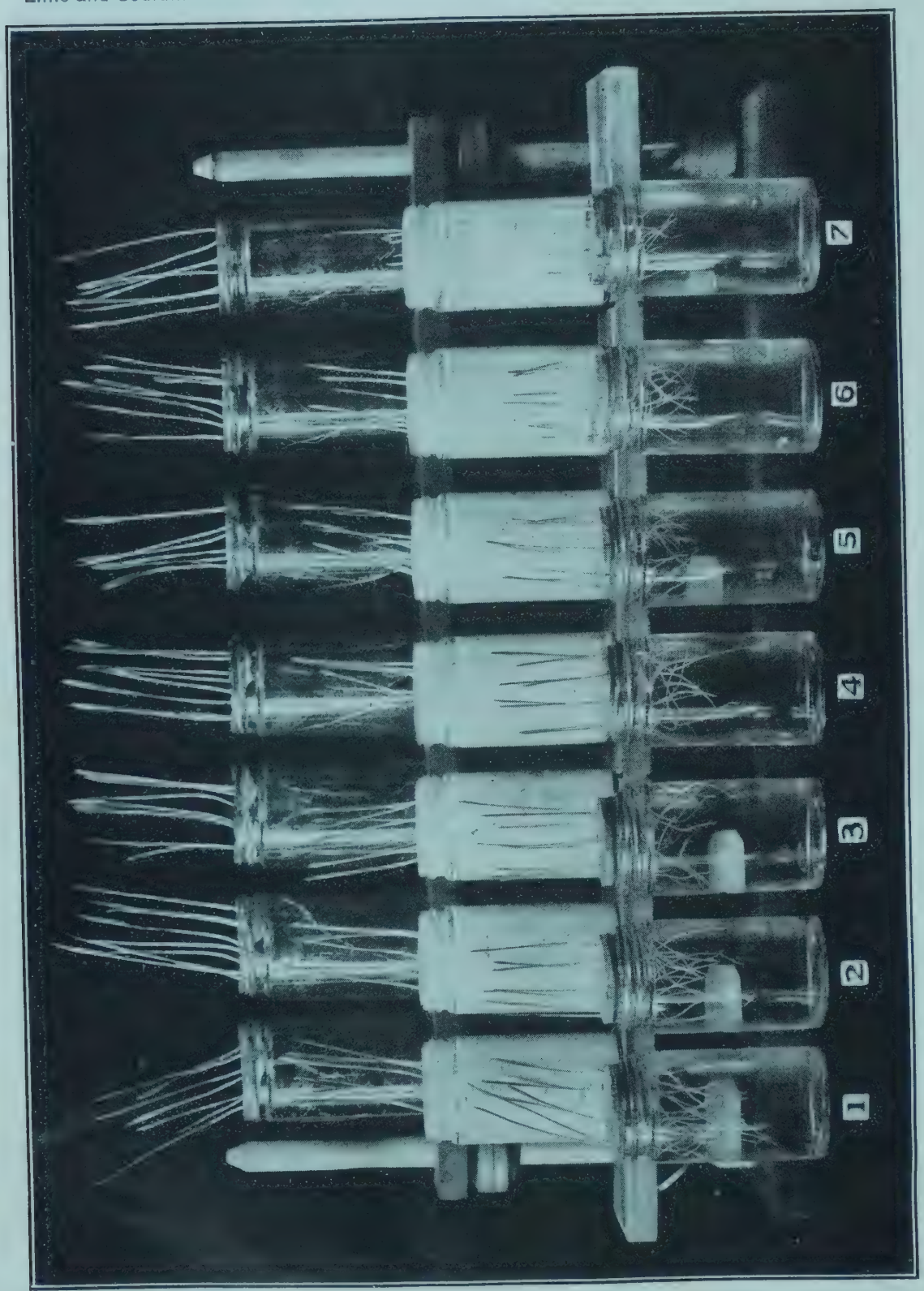
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PLATE 39

Seedlings grown in water, sand, and clay, showing effects of (1) distilled water, (2) 500 parts per million sodium-chlorid solution, (3) 1,000 parts per million sodium-chlorid solution, (4) 1,500 parts per million sodium-chlorid solution, (5) 2,000 parts per million sodium-chlorid solution, (6) 3,000 parts per million sodium-chlorid solution, and (7) 4,000 parts per million sodium-chlorid solution.

PLATE 40

A.—Seedling, 9 days old, grown in clay watered with (1) distilled water, (2) 500 parts per million sodium-chlorid solution, (3) 1,000 parts per million sodium-chlorid solution, (4) 1,500 parts per million sodium-chlorid solution, (5) 2,000 parts per million sodium-chlorid solution, (6) 3,000 parts per million sodium-chlorid solution, and (7) 4,000 parts per million sodium-chlorid solution.

B.—Seedlings, 3 days old, removed from (1) 4,000 parts per million sodium-chlorid solution, (2) sand watered with 4,000 parts per million sodium-chlorid solution, and (3) clay watered with 4,000 parts per million sodium-chlorid solution.

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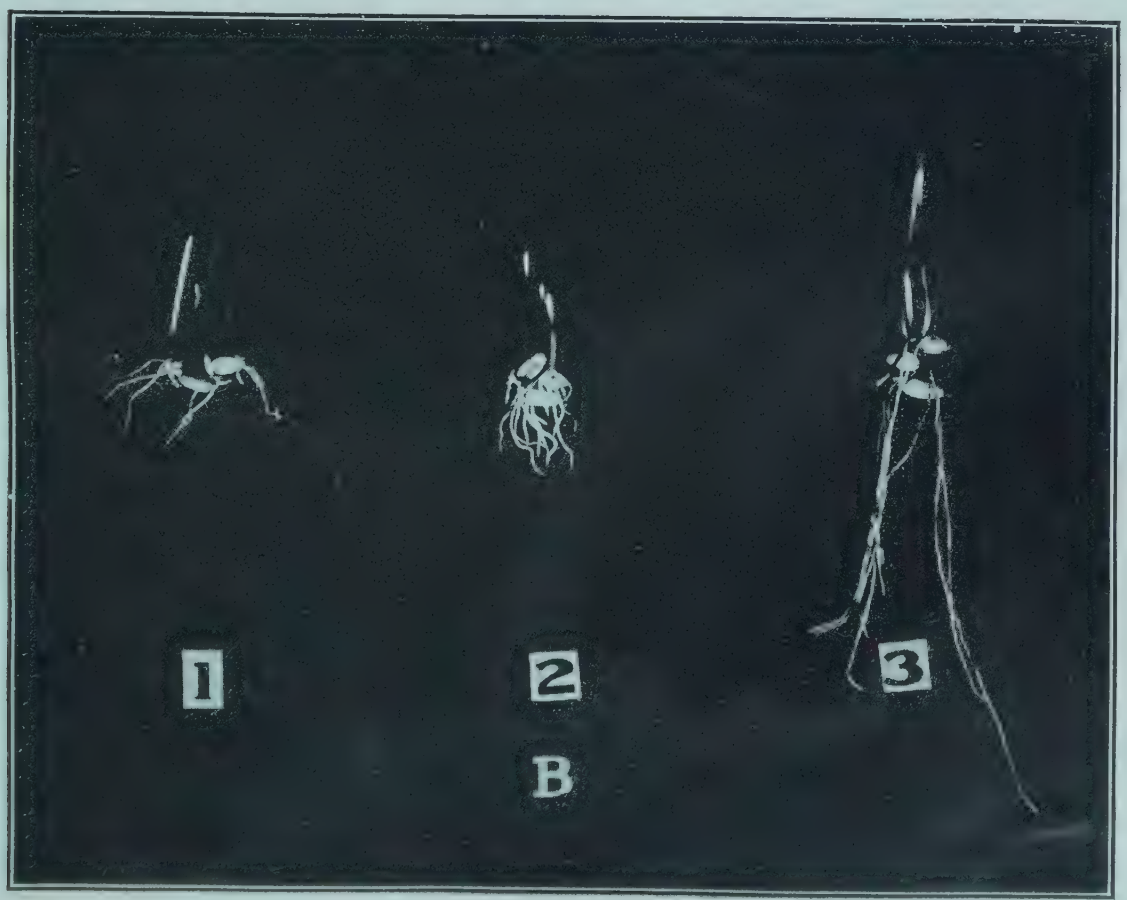
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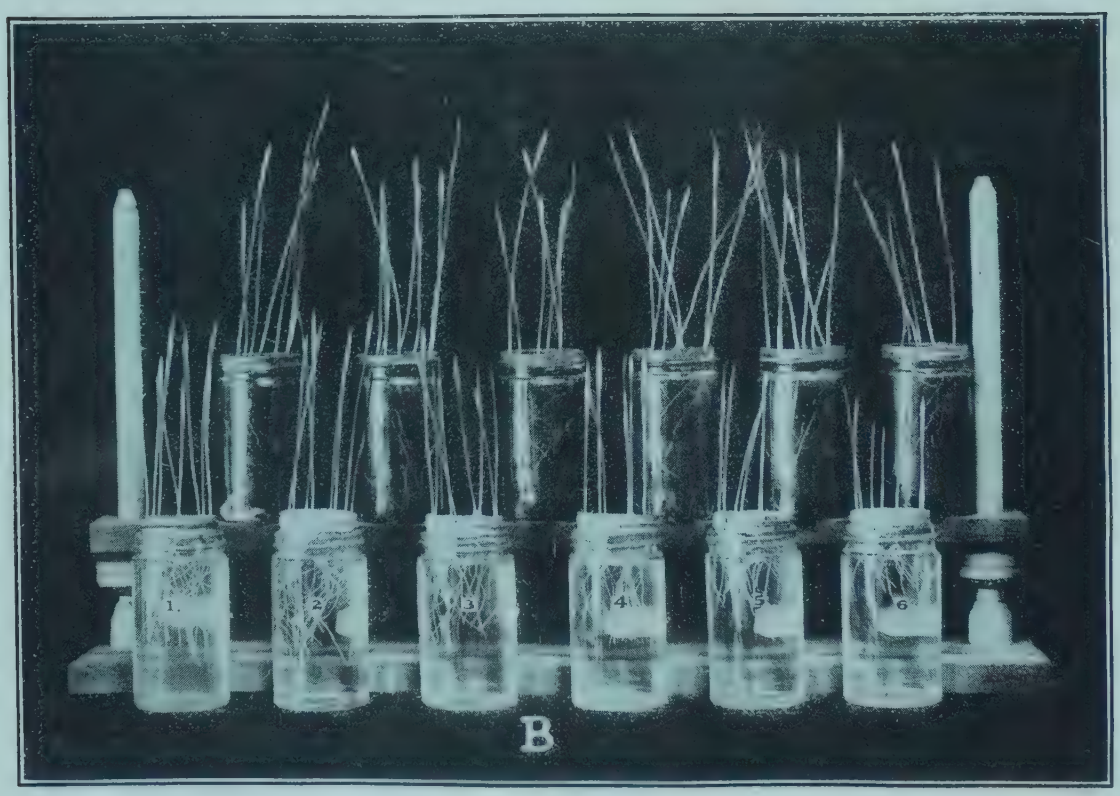
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PLATE 41

A.—Seedlings grown in sand and in the following solutions filtered through sand: (1) distilled water, (2) 1,000 parts per million of sodium chlorid, (3) 1,500 parts per million of sodium chlorid, (4) 2,000 parts per million of sodium chlorid, (5) 3,000 parts per million of sodium chlorid, and (6) 4,000 parts per million of sodium chlorid.

B.—Seedlings grown in clay and in the following solutions filtered through clay: (1) distilled water, (2) 1,000 parts per million of sodium chlorid, (3) 1,500 parts per million of sodium chlorid, (4) 2,000 parts per million of sodium chlorid, (5) 3,000 parts per million of sodium chlorid, and (6) 4,000 parts per million of sodium chlorid.

PLATE 42

A.—Seedlings, 10 days old, removed from sand watered with (1) distilled water, (2) 1,000 parts per million sodium-chlorid solution, (3) 1,500 parts per million sodium-chlorid solution, (4) 2,000 parts per million sodium-chlorid solution, (5) 3,000 parts per million sodium-chlorid solution, and (6) 4,000 parts per million sodium-chlorid solution.

B.—Seedlings, 10 days old, removed from clay watered with (1) distilled water, (2) 1,000 parts per million sodium-chlorid solution, (3) 1,500 parts per million sodium-chlorid solution, (4) 2,000 parts per million sodium-chlorid solution, (5) 3,000 parts per million sodium-chlorid solution, and (6) 4,000 parts per million sodium-chlorid solution.

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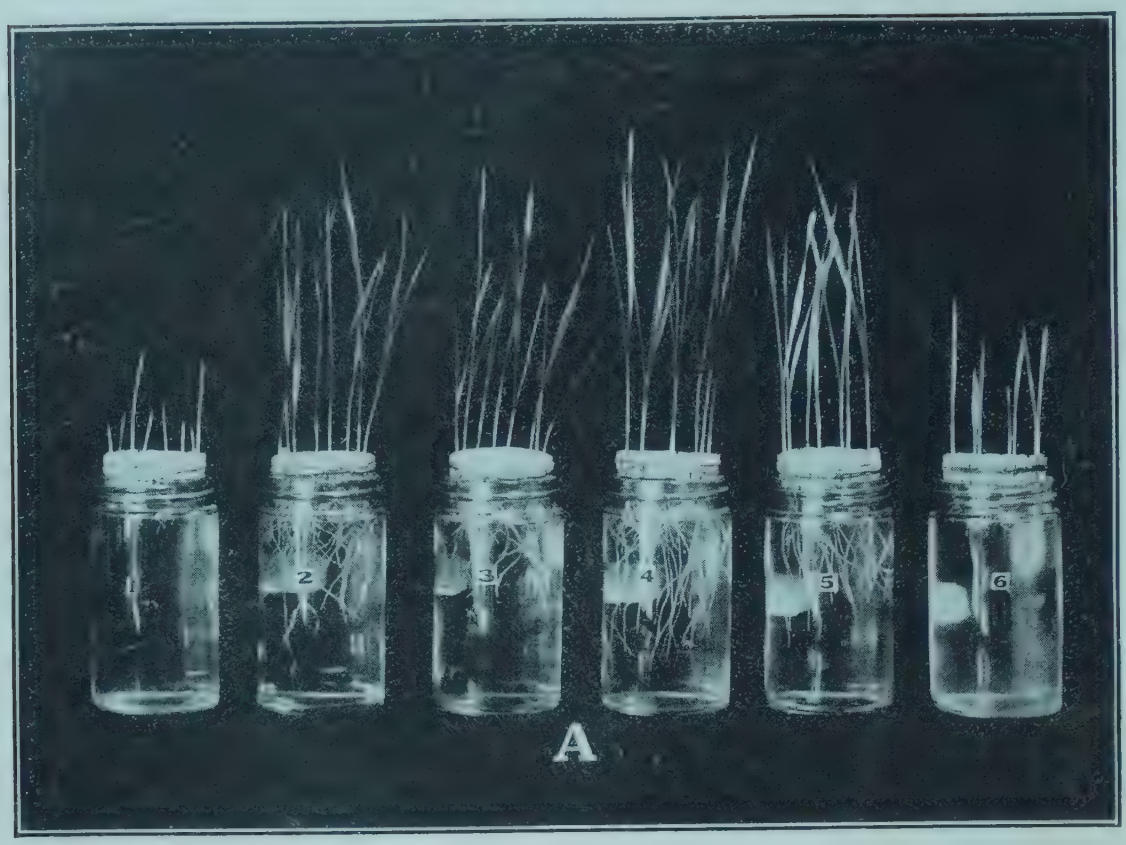
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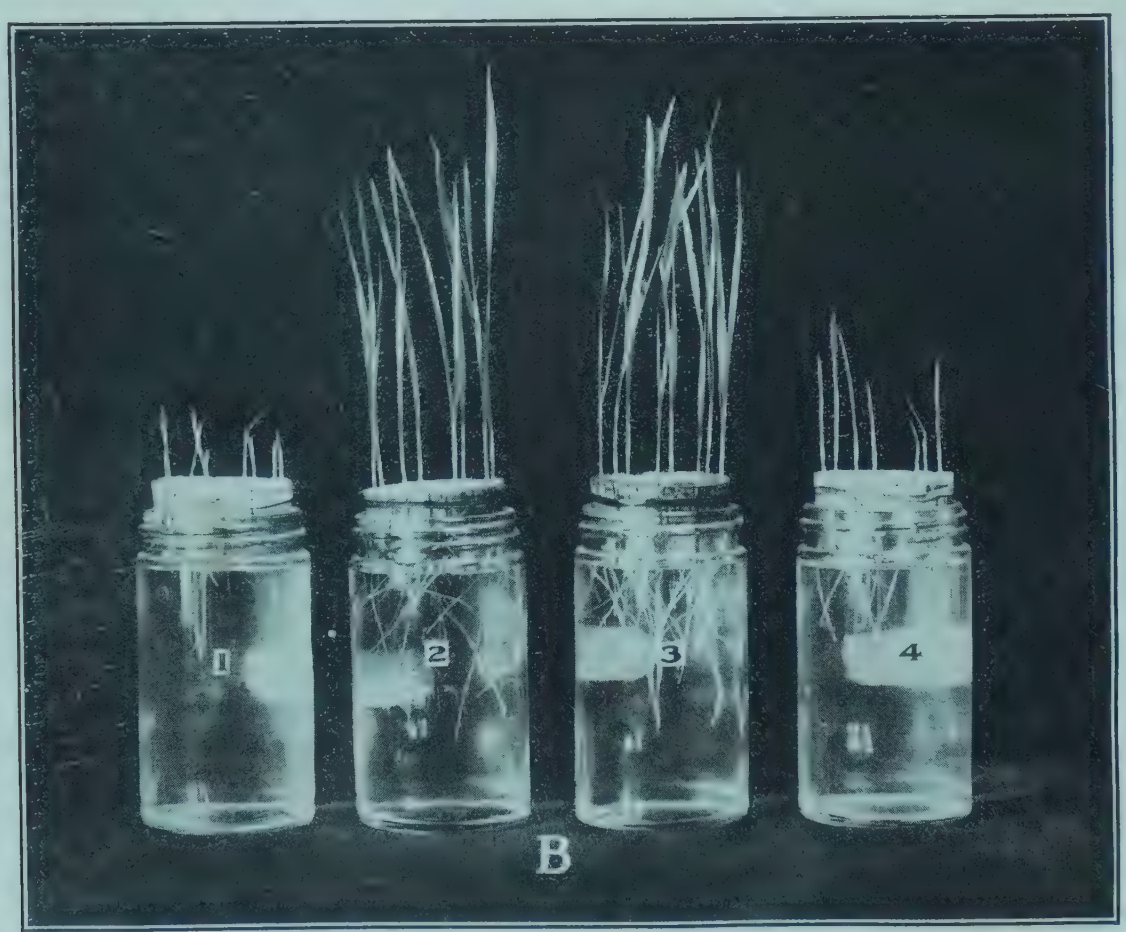
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PLATE 43

A.—Seedlings grown in (1) 4,000 parts per million sodium-chlorid solution, (2) 6,000 parts per million sodium-chlorid solution filtered through soil, (3) 8,000 parts per million sodium-chlorid solution filtered through soil, (4) 4,000 parts per million sodium-chlorid solution filtered through soil, (5) 4,000 parts per million sodium-chlorid solution added to distilled water that had previously been filtered through soil, and (6) 4,000 parts per million sodium-chlorid solution + 100 parts per million each of sodium nitrate, potassium chlorid, and sodium phosphate.

B.—Seedlings grown in (1) 4,000 parts per million sodium-chlorid solution, (2) 4,000 parts per million sodium-chlorid solution + 30 parts per million of calcium sulphate, (3) 4,000 parts per million sodium-chlorid solution + 30 parts per million of calcium oxid, and (4) 4,000 parts per million sodium-chlorid solution + 30 parts per million of magnesium bicarbonate.

153425°—20——2

PLATE 44

A.—Seedlings grown in (1) distilled water, (2) 4,000 parts per million sodium-chlorid solution + 30 parts per million of calcium sulphate, (3) 4,000 parts per million sodium-chlorid solution + 30 parts per million of calcium oxid, (4) 4,000 parts per million sodium-chlorid solution + 30 parts per million of magnesium sulphate, and (5) 4,000 parts per million sodium-chlorid solution + 30 parts per million of barium chlorid.

B.—Seedlings grown in (1) distilled water, (2) 4,000 parts per million sodium-chlorid solution + 30 parts per million of potassium chlorid, (3) 4,000 parts per million sodium-chlorid solution + 30 parts per million of sodium nitrate, (4) 4,000 parts per million sodium-chlorid solution + 30 parts per million of sodium phosphate, (5) 4,000 parts per million sodium-chlorid solution + 30 parts per million of ferric chlorid, and (6) 4,000 parts per million sodium-chlorid solution + 30 parts per million of potassium alum.

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PLATE 45

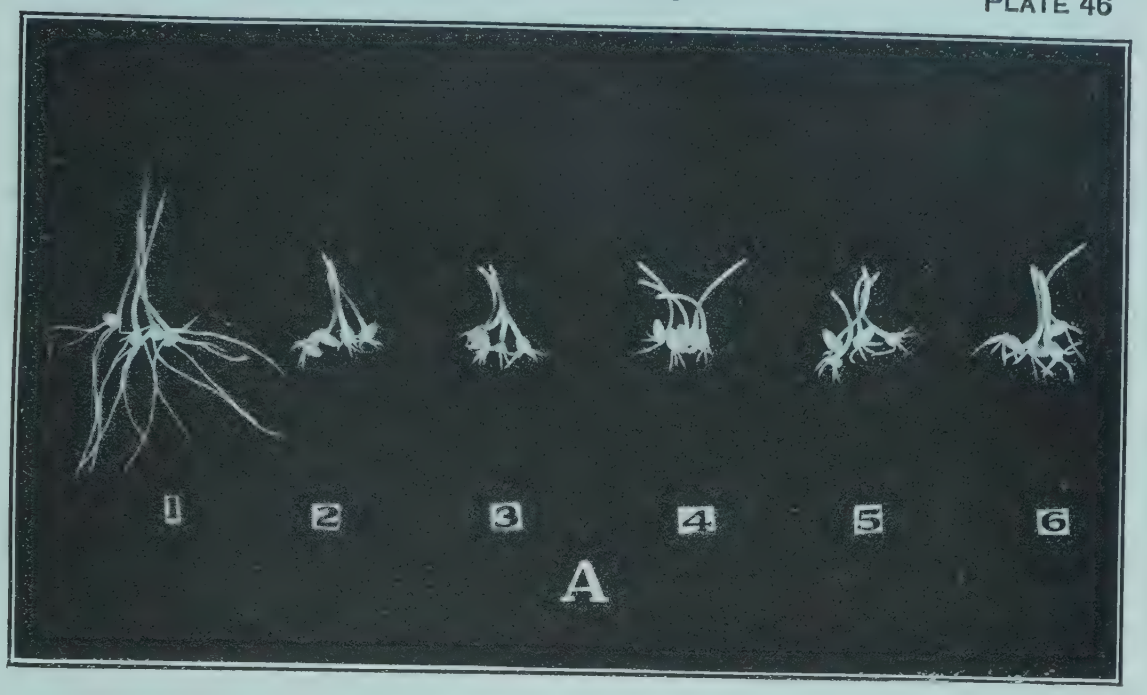
A.—Seedlings grown in sand watered with (1) 4,000 parts per million sodium-chlorid solution and (2) 4,000 parts per million sodium-chlorid solution + 30 parts per million of calcium sulphate.

B.—Seedlings grown in (1) 4,000 parts per million sodium-sulphate solution, (2) 4,000 parts per million sodium-sulphate solution + 30 parts per million of calcium sulphate, (3) 4,000 parts per million sodium-sulphate solution + 30 parts per million of calcium oxid, (4) 4,000 parts per million sodium-sulphate solution + 30 parts per million of magnesium sulphate, and (5) 4,000 parts per million sodium-sulphate solution + 30 parts per million of barium chlorid.

PLATE 46

A.—Seedlings, 3 days old, grown in (1) distilled water, (2) 4,000 parts per million sodium-sulphate solution, (3) 4,000 parts per million sodium-sulphate solution + 30 parts per million of potassium chlorid, (4) 4,000 parts per million sodium-sulphate solution + 30 parts per million of sodium nitrate, (5) 4,000 parts per million sodium-sulphate solution + excess ferric hydrate, and (6) 4,000 parts per million sodium-sulphate solution + aluminum hydrate.

B.—Seedlings, 11 days old, grown in (1) 2,500 parts per million sodium-bicarbonate solution, (2) 2,500 parts per million sodium-bicarbonate solution + 30 parts per million of sodium nitrate, (3) 2,500 parts per million sodium-bicarbonate solution + 30 parts per million of potassium chlorid, (4) 2,500 parts per million sodium-bicarbonate solution + 30 parts per million of magnesium sulphate, and (5) 2,500 parts per million sodium-bicarbonate solution + 30 parts per million of calcium oxid.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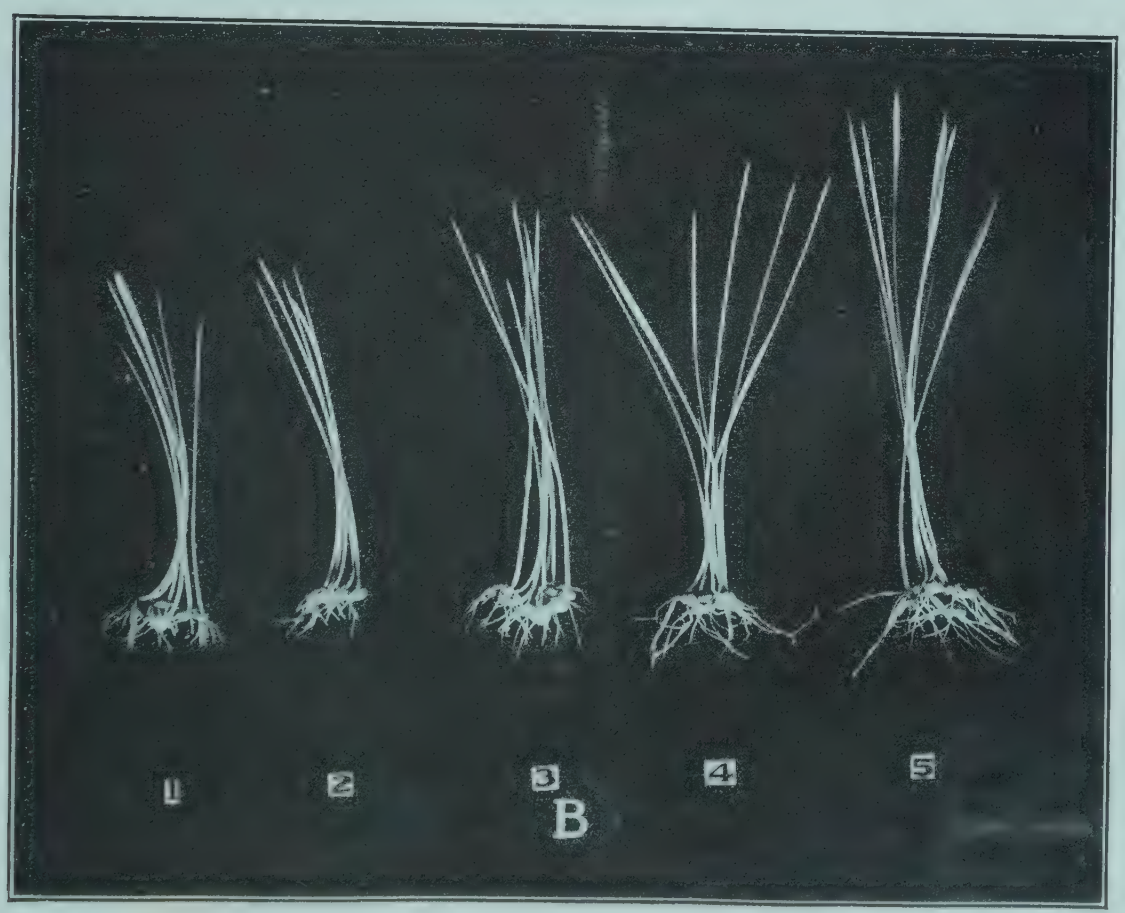
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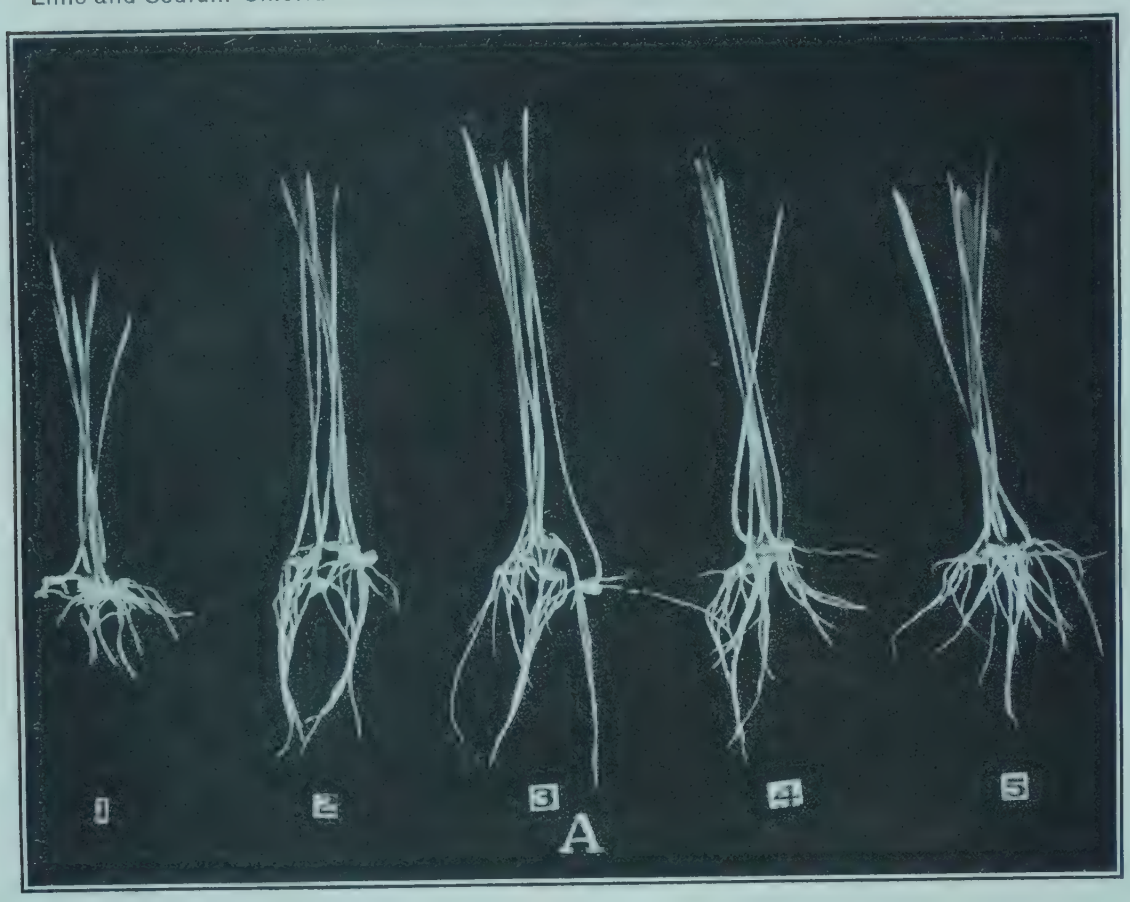
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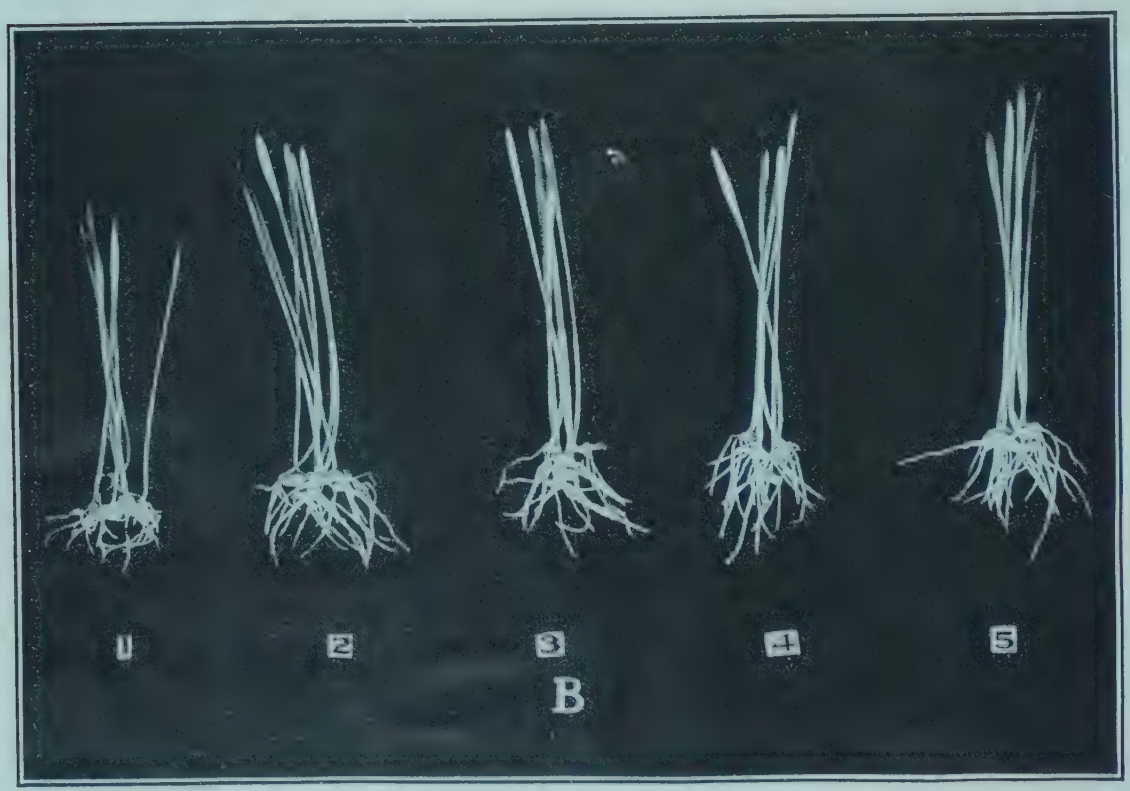
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PLATE 47

A.—Seedlings grown in (1) 4,000 parts per million sodium-chlorid solution, (2) distilled water, (3) 4,000 parts per million sodium-chlorid solution + 40 parts per million of calcium oxid, (4) 4,000 parts per million sodium-chlorid solution + 30 parts per million of calcium oxid, and (5) 4,000 parts per million sodium-chlorid solution + 20 parts per million of calcium oxid.

B.—Seedlings grown in (1) 4,000 parts per million sodium-chlorid solution, (2) 4,000 parts per million sodium-chlorid solution + 15 parts per million of calcium oxid, (3) 4,000 parts per million sodium-chlorid solution + 10 parts per million of calcium oxid, (4) 4,000 parts per million sodium-chlorid solution + 2 parts per million of calcium oxid, and (5) 4,000 parts per million sodium-chlorid solution + 1 part per million of calcium oxid.

RELATION OF MOISTURE IN SOLID SUBSTRATA TO PHYSIOLOGICAL SALT BALANCE FOR PLANTS AND TO THE RELATIVE PLANT-PRODUCING VALUE OF VARIOUS SALT PROPORTIONS

By JOHN W. SHIVE

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INTRODUCTION

Soil moisture is perhaps the most important single factor affecting crop production, and the relation of soil moisture to plant growth in general has been the subject of much investigation. Not so much attention has been given, however, to the relation of moisture in soils or other solid substrata to physiological salt balance or to the plant-producing value of complete fertilizer rations for plants, for the reason perhaps that adequate methods for quantitative studies were not available.

The need of some method by which the effects of nutrient solutions of known composition upon the growth of plants may be studied in the presence of some solid substratum resembling soil, but without so many of the biological and chemical complications always encountered in soil cultures, has practically been realized in the new sand-culture method recently developed by McCall (*8*).[1] By this method plants may be grown in sand supplied with nutrient solutions of any desired composition, which may be renewed or modified almost as readily as water cultures. This method makes it possible to study quantitatively the influence of various degrees of moisture in solid substrata upon the physiological salt balance, so far as this affects plant growth.

Harris (*5*) has pointed out that the effect of a fertilizer upon the growth of wheat is largely dependent upon the amount of soil moisture and emphasizes the point that fertilizer experiments, in order to be of any value, must be made under widely varying moisture conditions. It has been shown by Gile (*3*), McCool (*10*), Tottingham (*15*), McCall (*9*), Ayres (*1*), and others that the physiological value of any set of salt proportions varies, in general, with the total concentration of the medium; but as yet no general rule has been formulated to express the manner of this variation. It thus appears that physiological salt balance in nutrient solutions is largely dependent upon total concentration.

[1] Reference is made by number (italic) to "Literature cited," p. 377–378.

It has frequently been observed that plants grow less rapidly in a nutrient solution of high total concentration (within certain limits) without toxic effects than they do in a less concentrated solution containing the same constituents in the same relative proportions. It is also a fact of common observation that, regardless of the total concentration of the medium, plants grow less rapidly in a solid substratum, such as soil with low moisture content, than they do in the same medium with an optimum supply of moisture. If, now, a given set of salt proportions, in solution with approximately optimum total concentration for plant growth, should be added to an inert solid medium, such as sand, in such quantities as to produce a very low moisture content, it is obvious that plants would grow less rapidly in such a culture than in a similar culture supplied with the same solution to give an optimum moisture content. Thus, with any given set of salt proportions somewhat similar changes in growth or other forms of plant activity may result (1) from growing the plants in solutions with varying total concentrations of the salts in the given proportions, or (2) from growing the plants in an inert solid substratum, such as sand, supplied with the given set of salt proportions in solutions with constant total concentration so as to produce varying degrees of moisture content. Here any changes in growth with alterations in the degree of moisture would take place independently of the total concentration of the soil (sand) solution.

Since both variations in total concentration of the nutrient medium and alterations in the moisture content of a solid substratum have a somewhat similar influence upon plant activities with respect to retarded or accelerated growth rates, and since it is already well known that the physiological value of any given set of salt proportions is markedly influenced by total concentration, it seemed desirable to determine whether or not the salt balance of nutrient solutions could be similarly influenced or the relative value of a fertilizer treatment be altered by variations in the degree of the moisture content of a solid substratum, such as sand, when the mineral nutrients are diffused as films on the solid particles in the form of solutions of constant total concentrations. The following pages present the result of an experimental study dealing with this question.

OUTLINE OF EXPERIMENTAL METHODS

In order to study the effect of differences in the moisture content of a solid substratum upon the physiological salt balance of the nutrient medium, sand to which nutrient solutions were added was here used as the medium in which the plants were grown. The nutrient media added to the sand consisted of the 36 different 3-salt solutions (osmotic concentration value 1.75 atmospheres) comprised in an optimum series previously used with wheat (*12*) and with buckwheat (*13*) in studies of physiological salt balance. Three series of these sand cultures were

conducted simultaneously. The three series were alike in every respect except in the quantity of solution which was diffused as a film over the solid sand particles. All the cultures of the same series received the same amount of solution and were kept at approximately the same moisture content; but the cultures of the different series received different amounts of solution, so that the moisture content throughout each series was different from that of the other two series. Thus, corresponding cultures of the three different series received different amounts of the same solution, the differences in their moisture content being regulated by adding more or less solution as the case required.

All the cultures of one series were prepared with a moisture content of approximately 40 per cent of the water-holding capacity of the sand, all the cultures of another series with a moisture content of 60 per cent, and those of the third series with a moisture content of 80 per cent. These values were chosen as the result of preliminary tests which showed that a moisture content of 60 per cent of the water-retaining capacity of the sand used was well within the range for optimum growth, while the first and third values selected were considerably below and above this range.

The substratum used in these cultures consisted of white seashore sand which was thoroughly washed with tap water followed several times with distilled water. This sand had a water-retaining capacity of 25 per cent on the dry-weight basis (average of six tests) determined according to the method of Hilgard (*7, p. 209*). Thus the moisture contents of the three different series were 10 per cent, 15 per cent, and 20 per cent, respectively, based on the weight of the air-dry sand.

Half-gallon glazed earthenware pots were used as culture vessels. Each pot held 2,500 gm. of dry sand when filled to within several centimeters of the top. To prepare the sand in each pot for the planting of the seedlings, a sufficient amount of nutrient solution was poured into the sand to bring it almost to the point of saturation. Five carefully selected seedlings of spring wheat of the Marquis variety were then transplanted to the sand culture from a germinating net, after which the culture was flooded until free nutrient solution appeared over the surface of the sand to the depth of 1 cm. or more, thus fixing the seedlings in place and at the same time leveling the surface of the sand. This initial application required 750 cc. of solution for each culture. The excess solution was then withdrawn and the sand reduced to the desired moisture content by a method (*14*) which had previously been described and which is a modification of the method devised by McCall (*8*) for the renewal of solutions in sand cultures.

The solutions were renewed at 3-day intervals. After each culture had been restored to its original weight by the addition of distilled water, as much as possible of the old solution was withdrawn, and the culture was flooded with 500 cc. of new solution. The culture was then restored

to its original moisture content by withdrawing as much of the new solution as was required to accomplish this. To prevent loss of water by evaporation, all the cultures were sealed at the beginning of the experiment by pouring a thin layer of Briggs and Shantz wax (*2*) over the surface of the sand around the seedlings.

In all experiments of this kind it is, of course, practically impossible to maintain absolute uniformity in the moisture conditions of the substratum. This ideal condition may not even be very closely approximated because of transpirational water loss, which tends to decrease the moisture content of the cultures and at the same time to increase the total concentration of the solution. In the present work, however, excessive variation in the moisture content of the cultures and in the total concentrations of the solutions was prevented by the frequent addition of distilled water in quantities sufficient to restore the cultures to their original weights. The cultures were weighed daily, and during the later growth stages whenever the atmospheric conditions were such as to produce high rates of transpiration more frequent weighings were made and the original moisture conditions of the sand cultures were restored by the addition of distilled water. The highest water loss from any culture of the three series during the interval between two successive weighings was not greater than 4 per cent of the original volume of the solution present in the culture; or, on the dry-weight basis, this was not greater than 0.4 per cent for the series with the lowest moisture content, 0.6 per cent for the series with medium moisture content, and 0.8 per cent for the series with highest moisture content. This, of course, represents the extremes in the variations of the moisture conditions. Ordinarily the decrease in the moisture content of the cultures and the consequent increase in the total concentration of the solution during the intervals between two successive weighings were very much less than this. Small variations in the moisture conditions of the substratum, such as were encountered in these sand cultures, could scarcely be expected to have any material influence upon the physiological properties of the solutions as these affect the growth of the plants.

The three series of cultures here considered were conducted simultaneously for a period of 28 days after the seedlings had been transplanted to the sand cultures. The first triple series was conducted from November 30 to December 27, 1917. The second triple series, which was exactly like the first, was carried out between January 18 and February 15, 1918. At the end of the growth period the wax seals were removed from the cultures, the plants harvested in the usual manner, and the dry weights of the tops and roots obtained separately.

DISCUSSION OF RESULTS

YIELDS OF TOPS

For convenience in presenting the data, the three series of cultures here considered will be designated series A, series B, and series C, according as the cultures of the series were maintained at a moisture content of 40 per cent, 60 per cent, or 80 per cent of the water-retaining capacity of the sand. Since this triple series of cultures was repeated, two corresponding dry-weight measurements of both tops and roots are available for each culture; and by combining these, the average numerical data given in the tables were obtained.

Table I presents the average dry-weight yields of tops for each of the three moisture contents employed. The first column of each section gives the average absolute yield values in grams, while the second column gives the weights of tops relative to the weights from the first culture (R_1C_1) taken as unity. These relative dry-weight values were obtained by averaging the corresponding relative yield values from the two triple series, conducted during different time periods. Each of these relative data is, therefore, the average of two ratios and not the ratio obtained by dividing its corresponding average absolute dry-weight value by the average absolute value of the first culture (R_1C_1) in the same series. It thus happens that the relative values given in the table do not always bear exactly the same relation to each other as do the absolute values. The average relative data obtained from the group of cultures which produced the nine highest yields in each series (upper one-fourth) are shown in the table in italics, excepting the highest relative yield value of each series which appears in bold-face type. The culture numbers refer to the positions which the cultures occupy on the triangular diagrams graphically representing the variations in salt proportions and partial osmotic concentrations of the solutions added to the sand cultures.[1]

[1] For descriptions of this triangular diagramatic scheme see Shive (*12*), McCall (*9*), and Hibbard (*6*). An excellent discussion of the triangle system and its use in problems of plant nutrition has recently been published by Schreiner and Skinner (*11*).

TABLE I.—*Average dry weight of wheat tops grown 28 days in sand cultures supplied with 3-salt solutions, all having an osmotic concentration value of approximately 1.75 atmospheres*

Culture No.	Average dry weights of tops (5 plants).					
	Series A, 10 per cent moisture content.		Series B, 15 per cent moisture content.		Series C, 20 per cent moisture content.	
	Absolute.	Relative to R_1C_1 as unity.	Absolute.	Relative to R_1C_1 as unity.	Absolute.	Relative to R_1C_1 as unity.
	Gm.		*Gm.*		*Gm.*	
R_1C_1	0.1734	1.00	0.3211	1.00	0.3432	1.00
C_2	.4347	2.55	.5044	1.57	.3793	1.10
C_3	.3571	2.11	.5238	1.62	.4055	1.20
C_4	.4384	2.54	.5273	1.63	.3754	1.09
C_5	.4994	2.88	.5136	1.59	.4055	1.21
C_6	.4619	2.68	.5289	1.63	.4255	1.23
C_7	.4869	2.80	.4923	1.50	.4103	1.20
C_8	.4851	2.80	.4840	1.50	.4088	1.20
R_2C_1	.2887	1.97	.3647	1.15	.3680	1.12
C_2	.4739	2.77	.5196	1.60	.4350	1.32
C_3	.5352	3.06	.4858	1.49	.3893	1.18
C_4	.4793	2.74	.4750	1.48	.3780	1.14
C_5	.5772	*3.31*	.5285	1.63	.4161	1.23
C_6	.5164	3.05	.4654	1.44	.3630	1.20
C_7	.4622	2.66	.4390	1.36	.3556	1.03
R_3C_1	.3330	1.97	.4091	1.26	.4069	1.22
C_2	.4496	2.60	.5363	1.63	.3993	1.13
C_3	.5793	*3.36*	.5392	1.66	.4320	1.28
C_4	.4919	2.82	.4354	1.35	.4414	1.32
C_5	.5075	*3.41*	.4782	1.49	.3656	1.08
C_6	.4371	2.45	.4192	1.31	.3336	1.02
R_4C_1	.3475	2.05	.4220	1.32	.4303	1.30
C_2	.5256	3.06	.5623	1.76	.4632	1.37
C_3	.4879	2.82	.6756	*2.07*	.4786	*1.43*
C_4	.5615	*3.22*	.5931	1.83	.4236	1.27
C_5	.5275	3.04	.6353	*1.96*	.3804	1.15
R_5C_1	.4095	2.37	.4687	1.48	.4618	1.38
C_2	.6390	**3.67**	.8354	**2.58**	.6001	*1.75*
C_3	.6240	*3.56*	.7033	*2.17*	.5874	**1.76**
C_4	.5856	*3.37*	.6852	*2.13*	.4778	1.43
R_6C_1	.4705	2.78	.5391	1.67	.4915	*1.47*
C_2	.5666	*3.29*	.6645	*2.03*	.4903	*1.44*
C_3	.5402	*3.13*	.6072	*1.88*	.5475	*1.62*
R_7C_1	.4510	2.68	.6015	*1.87*	.5415	*1.59*
C_2	.5114	2.95	.6398	*1.99*	.5502	*1.62*
R_8C_1	.4302	2.53	.5727	1.79	.5213	*1.55*

Table II presents the average data of root yields corresponding to those of top yields in Table I.

TABLE II.—*Average dry weight of wheat roots grown 28 days in sand cultures supplied with 3-salt solutions, all having an osmotic concentration value of approximately 1.75 atmospheres*

Culture No.	Average dry weights of tops (5 plants).					
	Series A, 10 per cent moisture content.		Series B, 15 per cent moisture content.		Series C, 20 per cent moisture content.	
	Absolute.	Relative to R1C1 as unity.	Absolute.	Relative to R1C1 as unity.	Absolute.	Relative to R1C1 as unity.
	Gm.		*Gm.*		*Gm.*	
R1C1	0.0903	1.00	0.1750	1.00	0.1546	1.00
C2	.2013	2.27	.2475	1.40	.1895	1.22
C3	.1829	2.10	.2645	1.50	.1972	1.26
C4	.2263	2.54	.2893	1.64	.1860	1.17
C5	.2332	2.63	.2506	1.41	.2004	1.29
C6	.2056	2.33	.2698	1.53	.2031	1.28
C7	.2129	2.42	.2503	1.44	.2034	1.28
C8	.2153	2.42	.2414	1.36	.2235	1.42
R2C1	.1475	1.69	.1964	1.12	.1672	1.08
C2	.2322	2.57	.2528	1.42	.2013	1.30
C3	.2247	2.53	.2600	1.45	.1815	1.19
C4	.2141	2.46	.2279	1.29	.1777	1.17
C5	.2566	*2.90*	.3173	1.78	.2028	1.33
C6	.2481	*2.87*	.3296	*1.84*	.2259	1.46
C7	.2360	2.68	.2920	1.68	.2072	1.31
R3C1	.1622	1.82	.2196	1.24	.1855	1.21
C2	.1965	2.17	.2378	1.33	.1757	1.12
C3	.2225	2.45	.2823	1.58	.1963	1.26
C4	.2174	2.39	.2597	1.47	.2221	1.42
C5	.2745	*2.98*	.3165	*1.81*	.2196	1.37
C6	.2363	2.54	.3254	*1.87*	.1885	1.18
R4C1	.1708	1.89	.2267	1.29	.1693	1.10
C2	.2159	2.39	.2879	1.62	.2021	1.31
C3	.2285	2.51	.3023	1.69	.1982	1.26
C4	.2619	*2.96*	.3239	*1.84*	.2378	*1.50*
C5	.2824	**3.17**	.3998	**2.26**	.2131	1.38
R5C1	.1813	1.99	.2077	1.19	.1975	1.28
C2	.2542	*2.77*	.3439	*1.93*	.2409	*1.53*
C3	.2545	*2.76*	.3231	*1.81*	.2609	*1.68*
C4	.2663	*2.94*	.3603	*2.06*	.2860	**1.84**
R6C1	.2002	2.24	.2455	1.39	.1900	1.24
C2	.2365	2.62	.3012	1.69	.2443	*1.53*
C3	.2436	*2.69*	.3370	*1.94*	.2431	*1.56*
R7C1	.1769	2.04	.2685	1.52	.2599	*1.61*
C2	.2453	2.75	.2459	1.42	.2469	*1.58*
R8C1	.1904	2.13	.2769	1.58	.2392	*1.52*

The relative yield values of tops and of roots, taken directly from the proper columns of averages in the tables, were plotted on the triangular diagrams, as was done in earlier publications and by other writers. Since, however, the medium and low yields are of little interest in this connection, only that group of nine cultures in each series which produced the highest yields (upper one-fourth) will be considered in this discussion. On the diagrams of figure 1 areas are outlined to show the distribution of the 9 highest yield values of tops for each of the three different series of 36 cultures. The region or regions in each diagram including these nine high-yielding cultures are indicated by shaded areas. The highest-yielding culture in each series is marked by a circle.

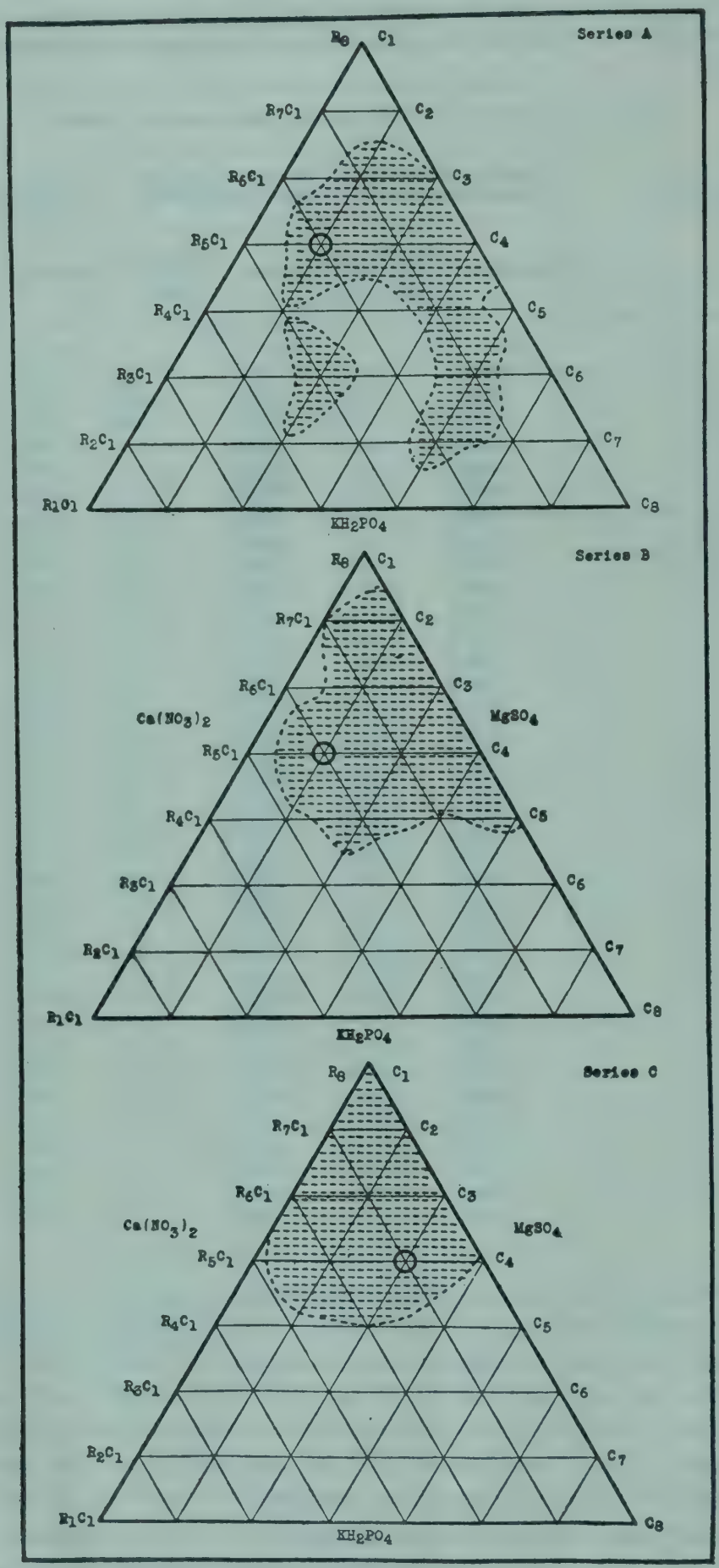

FIG. 1.—Diagrams showing the position of the cultures producing the nine highest yields of wheat tops in each series. The cultures giving maximum yields are marked by circles.

From a comparison of the three diagrams of figure 1 it is at once apparent that there is a marked degree of similiarity between the diagrams with respect to the locations of the areas of good growth. Out of a group of nine cultures in each series giving high yield values, five are corresponding cultures of the three series and are included in the areas marking good growth in each of the three diagrams. These five cultures are R_4C_4, R_5C_2, R_5C_3, R_6C_2, and R_6C_3. The two cultures R_7C_1 and R_7C_2 are also included in the group of high-yielding cultures in both series B and C, as is indicated on the diagrams representing these two series.

The highest average dry-weight yields obtained from series A and from series B were produced by corresponding cultures (R_5C_2) of the two series. The highest average relative yield obtained from series C was that produced by culture R_5C_3. It is to be noted, however, that during the two experimental periods of this series cultures R_5C_2 and R_5C_3 produced corresponding dry-weight yields which were nearly equal in value, so that the average absolute yield from the former was somewhat higher than that from the latter, while the average relative yield from the latter was slightly higher than that of the former.

A careful comparison of the diagrams of the three yields with reference to the location of the high-producing cultures brings out the fact that these areas approach more closely to the apex of the triangle and recede correspondingly from the base as the moisture content of the cultures in the different series is increased. Thus, in series A, which was maintained at a moisture content of 40 per cent of the water-retaining capacity of the sand, the areas of high yields lie mainly in a central region of the triangle between the second row of cultures from the base and the third row from the apex. In series B, which had a 60 per cent moisture content, the area of high yields is centrally located, mainly between rows 4 and 7. While this area has receded considerably from the base of the triangle toward the apex as compared with the areas of high yields in series A, it does not extend entirely to the apex and just touches the left margin at culture R_7C_1. In series C, which had an 80 per cent moisture content, the area of high yields has receded still farther from the base of the triangle and extends entirely to the apex, bordering on both right and left margins.

From the foregoing facts it is at once clear that the differences in the degrees of moisture employed in the cultures of these 3 series had no apparent influence upon the physiological salt balance of the culture producing maximum yields of tops, since the salt proportions and the total concentrations of the soil (sand) solutions of the highest-yielding cultures of these series were approximately the same, and since the cultures occupied the same relative positions in their respective series with reference to the yields produced, as is indicated in Table I and on the diagrams of figure 1. The fact that out of each of the 3 groups

of 9 high-yielding cultures 5 are corresponding cultures of the 3 series, as is indicated on the diagrams representing high yields of tops, still further points to the conclusion that good salt balance of nutrient solutions for wheat tops is not markedly disturbed when the solutions are diffused as films on the solid particles of an inert substratum in such a manner as to produce even large differences in the degrees of moisture. It is to be noted, however, that there is a slight but general shifting of the physiological salt balance for the group of 9 high-yielding cultures, as a whole, with each increase in moisture content, from a position in the series characterized by lower partial concentrations of potassium phosphate (KH_2PO_4) to one of higher partial concentrations of this salt and correspondingly lower ones of calcium nitrate ($Ca(NO_3)_2$) and magnesium sulphate ($MgSO_4$). Thus with the 36 different sets of salt proportions here employed in sand cultures, and with approximately constant total concentrations of the nutrient media, the best physiological salt balance with the lowest moisture content used was also the best with the medium and with the highest moisture content of the solid substratum.

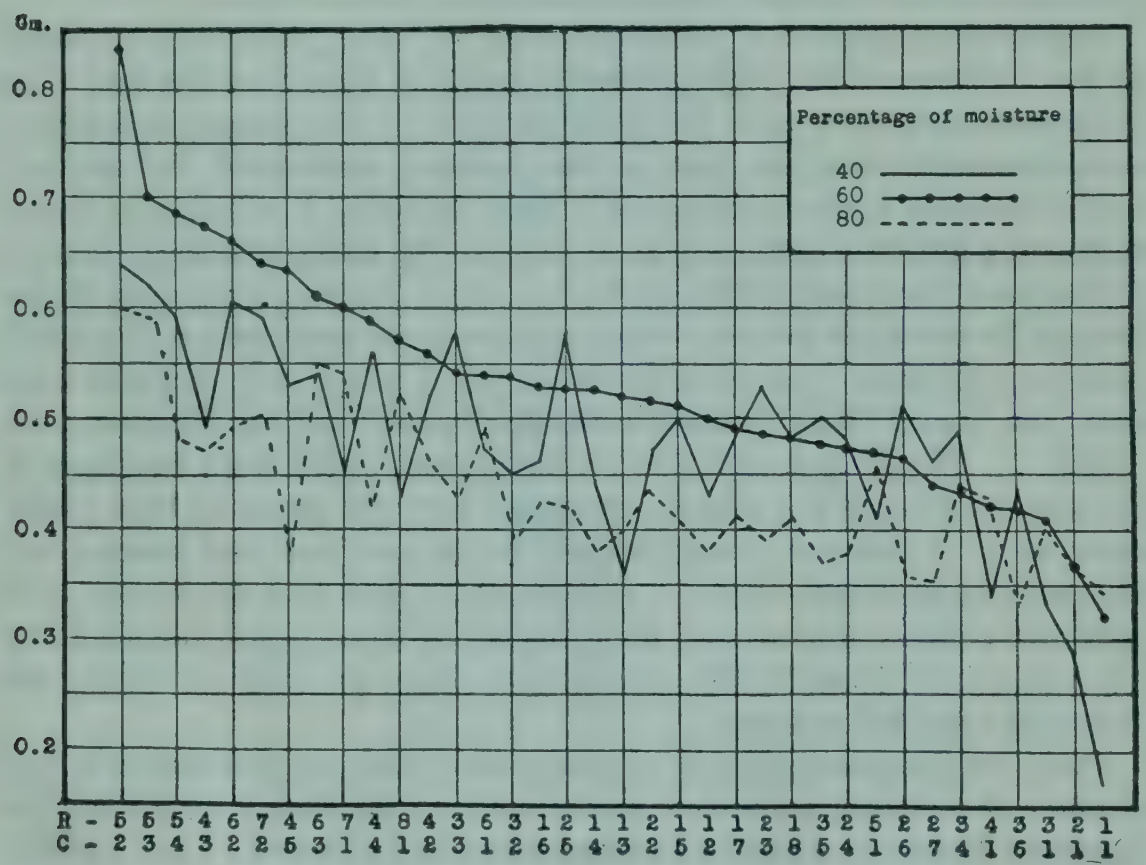

FIG. 2.—Average absolute yields of wheat tops for low, medium, and high moisture content of sand cultures.

The foregoing consideration of relative yields was not intended to show the effect of differences in degrees of moisture content upon the actual growth rates as indicated by the absolute dry-weight yields. To bring out the relation in question, the average absolute dry-weight yield values

of each series, taken from the columns of Table I, were arranged in the descending order of the values obtained from series B, employing a medium moisture content. These values were then plotted to form the three graphs shown in figure 2, representing the three different series of absolute dry-weight values. The continuous line marked by large dots and sloping somewhat uniformly downward to the right represents series B, with medium moisture content, while the broken line and the plain continuous line represent the yields obtained from the series with the highest and the lowest moisture contents, respectively.

Inspection of figure 2 shows that each of the three graphs has a decided tendency to slope downward to the right, thus indicating, in a general way, changes in the growth rates with variations in the salt proportions. In this respect the three series show a general agreement, which was brought out also by the triangular diagrams. The graph representing the series with medium moisture content lies above the other two graphs throughout most of its length. In the upper third of its course this graph shows the average absolute yields to be much higher than the corresponding yields from the other two series. However, that portion of the graph representing medium and low yields is intersected at various points by the other graphs. Nine yields from series A, with lowest moisture content, and three yields from series C, with highest moisture content, are thus shown to have higher values than the corresponding yields from series B.

The graphs of series A and series C are quite irregular and show little tendency toward parallelism. Practically the only tendency toward any agreement, in this respect, between these 2 series is shown for the first 5 cultures, which appear to rise and fall simultaneously. It will be observed, however, that more than two-thirds of the yields from series A are higher than the corresponding yields from series C. The highest yield from series C is lower than the highest from series A, and both are considerably lower than the highest yield from series B. In fact, the first 12 cultures of series B, as these cultures are arranged for the graphs of figure 2, show yield values which are considerably above the corresponding yield values of the other 2 series. This is somewhat striking in connection with the fact that series A and series C represent the extremes in the moisture content employed.

Perhaps the most important point brought out by the foregoing consideration of the absolute dry-weight values is the fact that the differences in the growth rates brought about by the variations in the moisture content, as indicated by the differences in the yield values of the corresponding cultures of the three series, are nearly as marked as are the differences in the rates of growth resulting from variations in the salt proportions throughout each series. This emphasizes the importance of a constant moisture supply in all pot-culture work of this kind, where the influence of relative salt proportions or of fertilizer treatments is

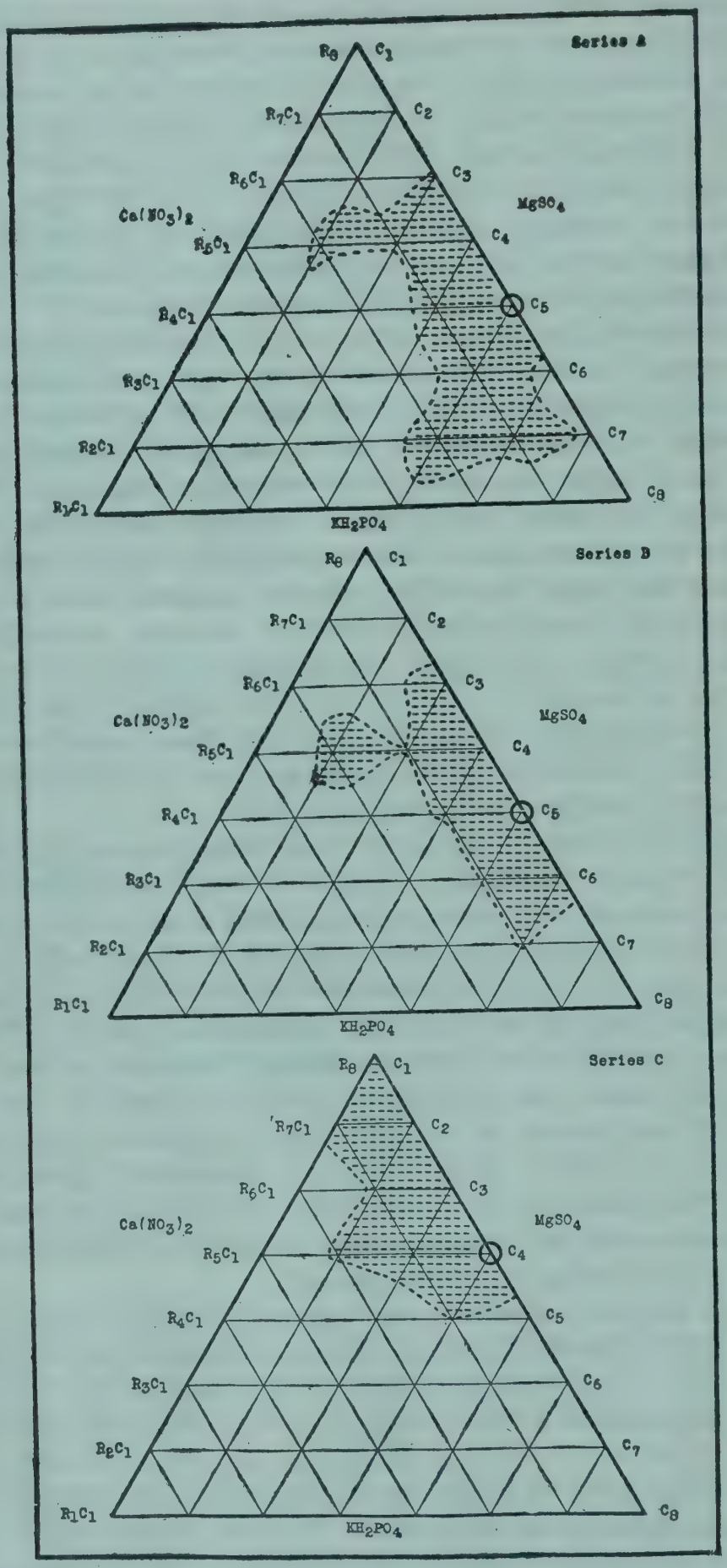

FIG. 3.—Diagrams showing the position of the cultures producing the nine highest yields of wheat roots in each series. The cultures giving maximum yields are marked by circles.

the factor under investigation. It appears that correct interpretations of the influence upon plant growth of such variables as the relative salt proportions here considered are not possible unless the moisture supply of the substratum in which the plants are rooted is maintained within very narrow variation limits.

YIELDS OF ROOTS

The position of the group of nine cultures which produced the highest average dry-weight yields of roots in each series is indicated by the shaded areas on the triangular diagrams of figure 3. This diagrammatic arrangement is in every respect similar to that of figure 1, which shows the distribution of high top yields. A comparison of the triangular diagrams of figure 3 shows the agreements between areas marking high root yields to be even more pronounced than are those between the corresponding areas representing the high yields of tops. Out of a total of nine cultures in each series which produced high yields of roots, five are corresponding cultures of the three series, as is indicated on the diagrams. These cultures are R_4C_4, R_5C_2, R_5C_3, R_5C_4, and R_6C_3. It will be observed also that eight corresponding cultures are represented in the areas of high root yields in both series A and series B. The highest yield of roots in each of these two series was obtained from culture R_4C_5, while in series C the highest yield was produced by culture R_5C_4.

Series A and series B, with low and medium moisture contents, respectively, show almost absolute agreement with respect to the location of the areas of high root yields. These areas occupy central positions on the right margins of the diagrams, while the corresponding area on the diagram of series C, with the highest moisture content, is shown to occupy a region on the right and left margins of the triangle, farther from the base and extending to the apex. It is thus evident that there is scarcely any shifting of the physiological balance of salt proportions for the group of nine cultures giving the highest yields of roots in passing from the low to the medium moisture content, but with the highest moisture content the physiological salt balance characterizing the nine high-yielding cultures shows the same tendency to migrate toward the apex of the triangle as did that of the nine cultures which produced high yields of tops in the same series. But this shifting of the physiological salt balance characterizing good yields of roots, with a change from the low to the high moisture content, is scarcely more pronounced than is that of the salt balance characterizing good top yields, since the same number of high root yields as of high top yields in each group of nine occurred with corresponding cultures in the three series.

The average absolute dry-weight yields of wheat roots for each of the three different degrees of moisture employed in the sand cultures are shown graphically in figure 4, in the same manner as were the corresponding yields of tops in the graphs of figure 2.

The dry-weight yields of the series employing a medium moisture content (series B) were arranged in the order of their values, beginning with the highest. These are represented in the upper graph of figure 4. The yield values of series A and series C were plotted in the same order,

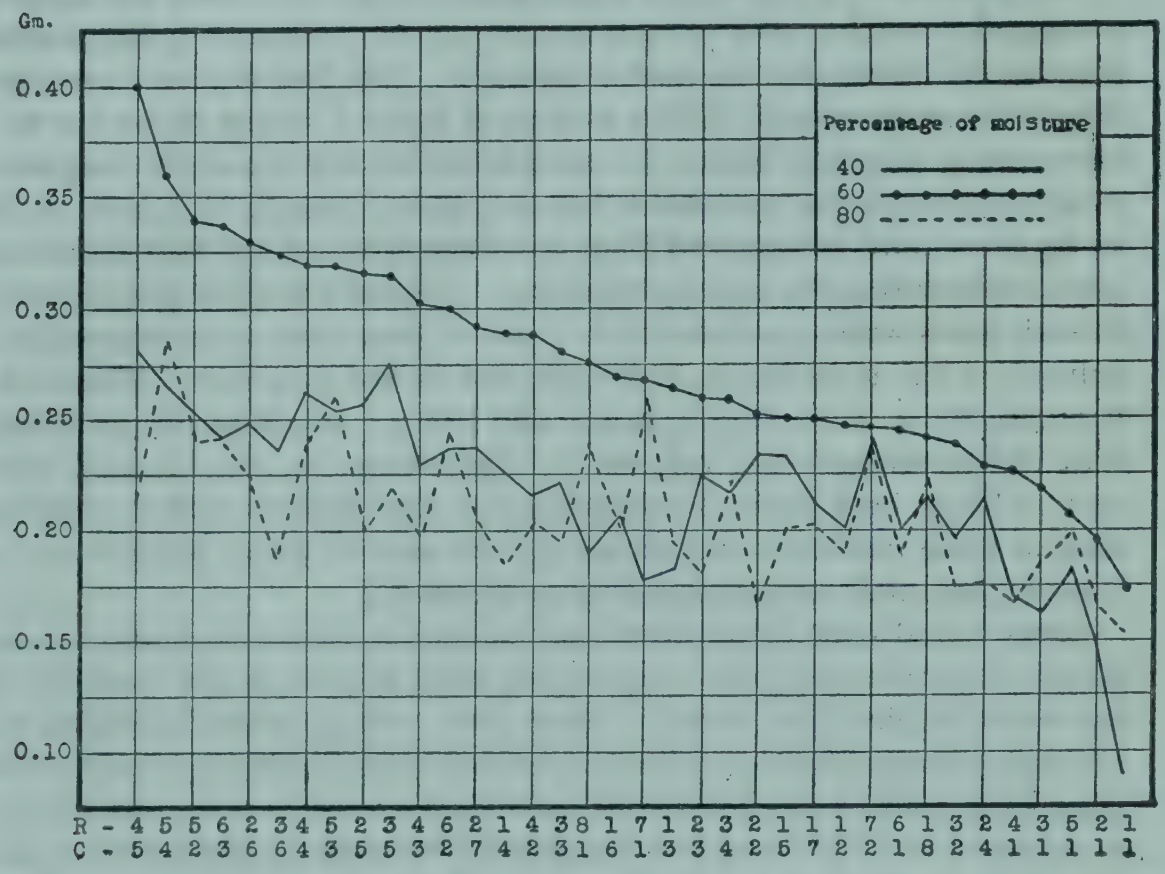

FIG. 4.—Average absolute yields of wheat roots for low, medium, and high moisture content of sand cultures.

and these are represented by the graphs indicated by the continuous and the broken line, respectively. As in the graphs representing the yields of tops, there is a decided tendency for each of these graphs to slope downward to the right, thus indicating the difference in the yield values brought about mainly by the variations in the salt proportions. But this tendency is much more marked in series B than it is in either of the other two series. The medium moisture content (series B) shows the highest absolute dry weights of roots throughout the entire series. It is to be noted also that the highest yields in the two series with the extremes in the moisture content employed are nearly equal in value and very much lower than the highest yield produced with medium moisture content.

The differences between the graphs representing the root yields from the two series of cultures with the lowest and the highest moisture content are not pronounced; these two series resemble each other with re-

spect to the actual root yields produced more than either one resembles the series with medium moisture content. This particular relation between these two series was also apparent but less marked in the graphs representing the dry-weight yields of tops; and, as previously remarked, it is of especial interest in connection with the fact that these two series represent the extremes of moisture content. The graphs of these two series intersect at various points, but there is no marked tendency for the yield values, as a whole, to be either higher or lower with one series than with the other. From an *a priori* consideration of the problem, however, this is not what might be expected. It appears that the growth rates of both tops and roots are considerably retarded by low moisture content. This is unquestionably the result of greater resistance to water absorption by the plant roots, resulting in an internal water supply deficient for optimum growth.

It might be expected that with sand cultures such as were here employed, with approximately constant total concentrations of the nutrient media, a progressive increase in the moisture content up to the point of saturation as a limit to the moisture variation would correspondingly accelerate the growth rates of the plants because of a decreased resistance to water absorption. This, however, does not occur, as the graphs of figures 2 and 4 clearly show. It appears that the growth rates are accelerated by an increase in the moisture content of the sand cultures up to a certain optimum, after which with further increase in the moisture content there is a marked retardation in the rates of growth. This is to be attributed to other factors unfavorable to growth, which are introduced with increased moisture content above the optimum. Whatever the nature of these factors may be, it is clear, as has been brought out, that a sand culture supplied with a well-balanced nutrient solution to give an optimum moisture content is much superior in plant-producing power to a similar sand culture supplied with the same solutions to produce a moisture content closely approaching the point of saturation. In this connection Hall, Brenchley, and Underwood (*4*) have pointed out that growth in nutrient solutions diffused as films over sand particles is much superior to that in water cultures with the same solutions, but the growth in water cultures is similarly increased when a continuous air current is passed through the solutions. They ascribe enormous advantages to the plants from continuous aeration and attribute to this factor alone the superiority of growth in solid substrata over that in the ordinary water cultures in which aeration is not continuous.

The fact that the average dry-weight yields of both tops and roots from the best nine cultures of the series employing a medium moisture content are always considerably higher than are the corresponding yields from the series with the lowest and highest moisture contents here used clearly shows that well-balanced solutions with optimum total concentrations for plant growth are not alone sufficient to produce the best

yields of which these solutions are capable when they are diffused as films on the particles of a solid substratum, such as sand, and in quantities in excess of the requirements of the plants. An optimum degree of moisture under such conditions is essential to impart to the soil (sand) solution its maximum physiological value. It appears that the actual plant-producing power of any given set of salt proportions or of any fertilizer treatment is largely determined by the moisture conditions of the substratum.

INFLUENCE OF MOISTURE CONTENT OF SAND CULTURES UPON TRANSPIRATION AND WATER REQUIREMENT

Throughout the growth period the transpirational water loss from each culture during the intervals between successive weighings was recorded. The total water loss from each culture was then determined by summing the partial losses thus recorded for the entire growth period. The ratio

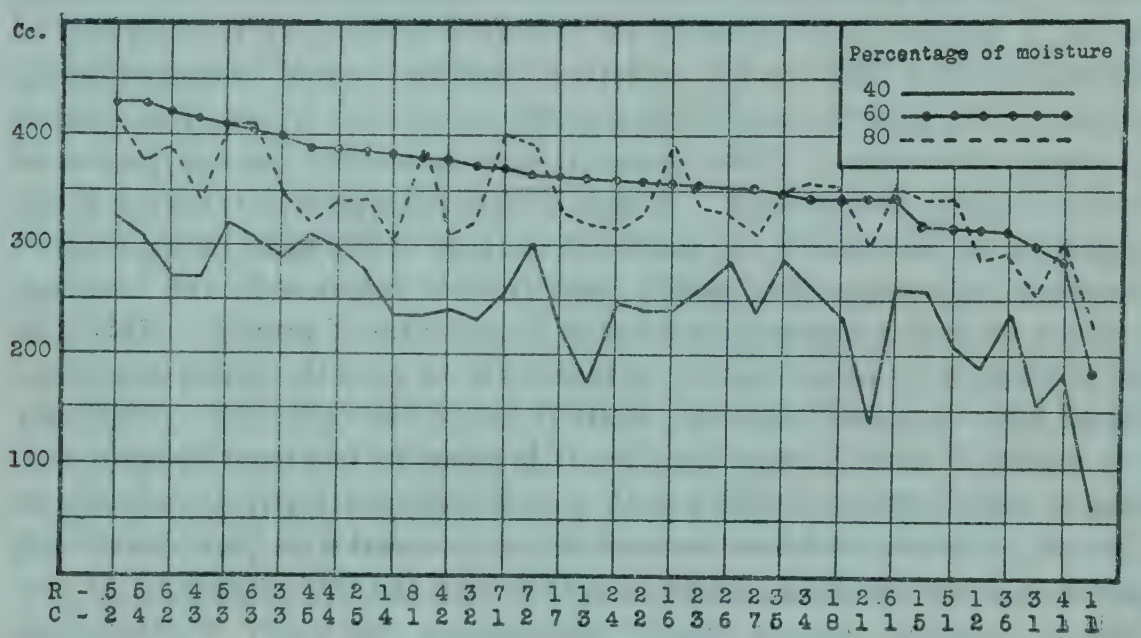

FIG. 5.—Amounts of water lost by transpiration from wheat plants grown in sand cultures with low, medium, and high moisture content.

between the amount of water lost by transpiration during the entire growth period and the dry weight of tops and of roots produced during the same period were calculated for each culture. These ratios, which are quantitative measures (expressed in cubic centimeters) of the water lost by transpiration during the production of a single gram of dry tops or dry roots, as well as the total water loss from each culture of the three series, are presented in Table III. Each value in the table represents the average of corresponding data obtained from duplicate pairs of cultures of the repeated series.

TABLE III.—*Transpirational water loss and amounts of water required for the production of each gram of tops and of roots (water requirement)*

Culture No.	Transpiration.			Water requirement.					
				Tops.			Roots.		
	Series A.	Series B.	Series C.	Series A.	Series B.	Series C.	Series A.	Serie B.	Series C.
	Cc.	*Cc.*	*Cc.*	*Cc.*[a]	*Cc.*[a]	*Cc.*[a]	*Cc.*[a]	*Cc.*[a]	*Cc.*[a]
R_1C_1	66	184	227	382	573	661	733	1,051	1,463
C_2	192	318	289	442	631	762	955	1,288	1,530
C_3	178	361	321	498	688	791	977	1,362	1,630
C_4	234	382	305	534	735	814	1,035	1,322	1,640
C_5	259	319	341	519	621	840	1,111	1,271	1,705
C_6	243	356	390	526	673	917	1,180	1,318	1,920
C_7	228	362	333	468	736	812	1,071	1,447	1,640
C_8	238	344	353	491	711	862	1,107	1,428	1,575
R_2C_1	143	244	300	395	668	815	967	1,246	1,795
C_2	242	357	328	510	688	754	1,042	1,411	1,632
C_3	258	355	334	482	730	858	1,146	1,365	1,835
C_4	249	358	316	520	753	836	1,164	1,571	1,776
C_5	274	386	341	475	731	820	1,067	1,217	1,680
C_6	286	354	330	554	761	910	1,153	1,213	1,460
C_7	241	352	312	521	802	877	1,022	1,205	1,508
R_3C_1	157	302	308	471	738	756	968	1,371	1,655
C_2	233	372	320	518	694	802	1,183	1,562	1,818
C_3	286	400	354	494	742	820	1,272	1,418	1,796
C_4	260	345	356	529	793	807	1,198	1,326	1,602
C_5	289	349	349	570	730	954	1,051	1,100	1,586
C_6	244	316	295	558	754	884	1,033	973	1,563
R_4C_1	185	288	307	532	682	713	1,083	1,270	1,815
C_2	242	378	353	460	673	762	1,120	1,312	1,750
C_3	270	415	344	552	614	718	1,180	1,375	1,738
C_4	299	390	336	532	658	792	1,141	1,204	1,412
C_5	311	394	321	590	620	845	1,102	985	1,508
R_5C_1	213	318	341	522	678	739	1,297	1,530	1,721
C_2	324	429	417	507	514	695	1,276	1,247	1,730
C_3	320	410	409	512	573	697	1,256	1,274	1,566
C_4	308	428	376	526	625	787	1,158	1,188	1,315
R_6C_1	262	344	356	556	638	724	1,310	1,398	1,872
C_2	270	416	392	476	627	800	1,138	1,383	1,607
C_3	305	406	406	565	669	741	1,250	1,206	1,670
R_7C_1	257	371	404	570	617	746	1,452	1,378	1,554
C_2	299	363	390	584	638	710	1,221	1,475	1,580
R_8C_1	236	380	384	550	664	737	1,243	1,372	1,608

[a] Quantity of water computed as required for production of 1 gm. of dry tops or roots.

In order to bring out the relation between the moisture content of the sand cultures and the amounts of water lost by transpiration the average actual amounts of water lost, as given in the table, were plotted to form the graphs of figure 5. These graphs, as well as those in figures 6 and 7 which show the water requirement, were prepared in the same manner as were those representing the yields of tops and of roots (fig. 2, 4). The actual average losses from the cultures of series B, which had a medium moisture content, arranged in the descending order of their magnitudes are represented by the full line marked by large dots and sloping uniformly downward to the right. The average losses from the cultures of the other two series were then arranged in the same order and were plotted on the same scale.

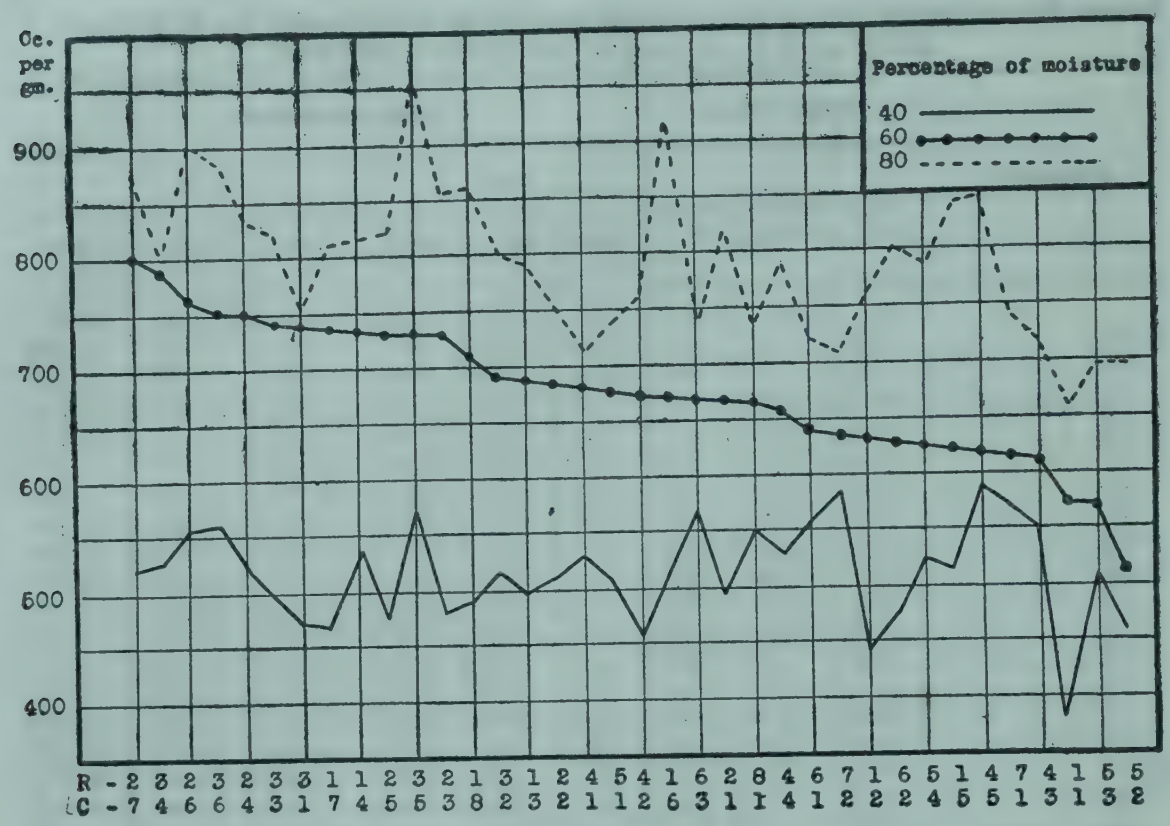

FIG. 6.—Water requirement (in cubic centimeters per gram) of wheat tops grown in sand cultures with low, medium, and high moisture content.

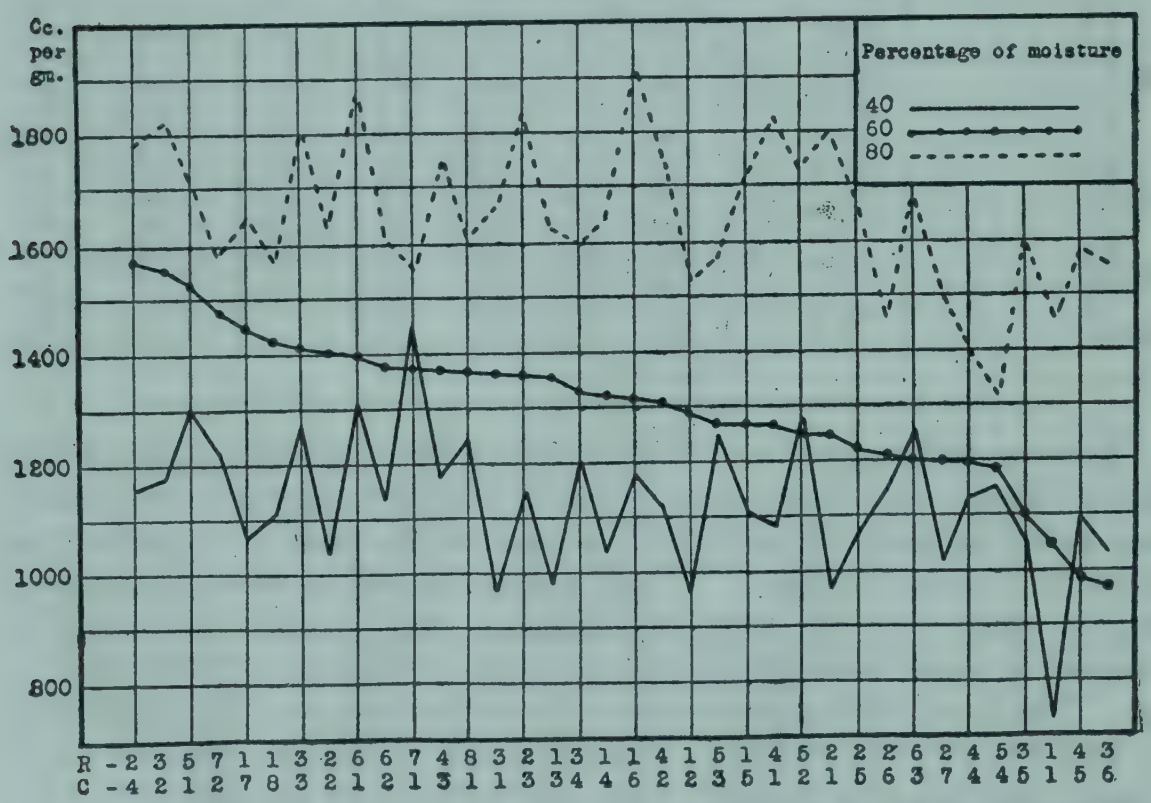

FIG. 7.—Water requirement (in cubic centimeters per gram) of wheat roots grown in sand cultures with low, medium, and high moisture content.

It will be observed that the cultures of the series having the lowest moisture content throughout the experiment exhibit a much lower water loss than do the corresponding cultures of the other two series. There is no such clearly defined relation, however, between the actual amounts of water lost and the highest and medium degrees of moisture employed with the cultures of series B and series C, respectively. There appears to be no general tendency for the transpiration to be either higher or lower with the medium moisture content than with the highest.

A comparison of the transpiration graphs with those representing the yields of tops and of roots (fig. 2, 4), brings out several interesting relations. Low moisture content of the sand cultures denotes low yields of tops and of roots and low transpiration, while a high moisture content corresponds to low yields of tops and of roots but high transpiration. Thus, while low soil (sand) moisture retards both the rate of growth and transpiration, excessive moisture of the substratum, on the other hand, markedly retards the growth rates but does not correspondingly retard transpiration. The medium moisture content, 60 per cent of the water-retaining capacity of the substratum, gave the highest yields of both tops and roots and the highest transpiration.

The water requirement of tops and of roots is graphically shown in figures 6 and 7, respectively, in the same manner as are the yields of tops and of roots and the actual amounts of water lost by transpiration (fig. 2, 4, 5).

Inspection of the graphs of water requirement of tops and of roots shows at once that the values of the water requirement ratios increase with each increase in the moisture content of the substratum. Thus, from the positions of the graphs it is clear that the values of the water requirement ratios are determined by the moisture conditions of the cultures. High, medium, and low moisture content of the substratum is correlated with high, medium, and low water requirement ratios, respectively, of both tops and roots. This relation is perfectly definite for the wheat tops of these tests and, in the main, also for the roots, although several cultures with the lowest moisture content (series A) show water requirement ratios which are slightly higher than are those of the corresponding cultures with medium moisture content (series B), as is indicated on the graphs of figure 7.

As has previously been pointed out, both the highest and the lowest moisture content here employed retarded the growth rates, the former through some harmful influence (perhaps insufficient aeration) related to excessive moisture, the latter undoubtedly through the resistance offered to water absorption by the plant roots, which resulted in an insufficient internal water supply necessary for good growth. The transpiration graphs show, however, that with the highest moisture content (series C) the transpiration rates were not correspondingly retarded, so that under these conditions a comparatively large amount

of water was required to produce a single gram of dry plant material, and the water requirement ratios are high for this series. With the lowest moisture content, on the other hand, the transpiration rates were retarded proportionately more than were the growth rates. Thus for each gram of dry plant substance produced a relatively small quantity of water was required, and the water-requirement ratios for this series are low. A further comparison of the graphs representing water requirement of tops and of roots with the corresponding ones of transpiration and yields brings out the fact that the approximately optimum moisture content here employed with the cultures of series B is correlated with maximum yields of tops and of roots, with high transpiration rates, and with water-requirement ratios which are intermediate in value between those of the other series (series A and C), having degrees of moisture in the sand cultures considerably below and above that of the optimum.

SUMMARY

This paper is a report of studies on the influence of different degrees of moisture in a solid substratum upon the physiological salt balance for young wheat plants and upon the relative plant-producing value of various salt proportions.

Three different degrees of moisture were maintained in sand cultures: 40 per cent, 60 per cent, and 80 per cent of the water-retaining capacity of the sand. Tests were made with 36 different sets of salt proportions of the three salts KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ in solutions (Shive's optimum 3-salt series) with each of the three different degrees of moisture. The solutions, all having an initial total osmotic concentration value of 1.75 atmospheres, were supplied to the sand cultures in such quantities as to produce the different degrees of moisture. All three of the different moisture-content series were conducted simultaneously and were then repeated. The culture solutions were renewed every third day. The cultures were weighed each day, and the water loss by transpiration was restored through the entire growth period of 28 days. The growth period was the same for the first and the repeated series.

The main results of this study may be summarized as follows:

(1) For the set of conditions under which these tests were made the physiological balance of the nutrient solutions producing the best yields of wheat tops and roots was not altered by variations in the moisture content of the solid substratum to which the solutions were applied. The physiological balance of salt proportions which was best with the lowest moisture content was the best also with the medium and the highest degree of moisture.

(2) A slight shifting of the physiological balance, as this affects the growth of plants, is indicated for the growth of 9 high-yielding cultures, as a whole, out of a series of 36, with each increase in the moisture

content of the cultures, from a position in the series characterized by lower partial concentration of KH_2PO_4 to one of higher partial concentration of this salt and correspondingly lower ones of $Ca(NO_3)_2$ and $MgSO_4$.

(3) Good physiological balance and optimum total concentration of a nutrient solution for plants is not alone sufficient to produce the best growth of which the solution is capable when it is diffused as a film on the particles of a solid substratum. An optimum degree of moisture, under such conditions, is essential to impart to the soil (sand) solution its maximum physiological value. The actual plant-producing value of any fertilizer treatment is thus largely determined by the moisture conditions of the substratum.

(4) The lowest degree of moisture here employed with sand cultures is correlated with low yields of tops and of roots, with the lowest transpiration rates, and with the lowest water requirement ratios. The highest moisture content of the cultures, on the other hand, is associated with low yields of tops and of roots, with high transpiration rates, and with the highest water requirement ratios. The medium degree of moisture, which is approximately optimum for the substratum here used, is correlated with the highest yields of tops and of roots, high transpiration rates, and medium water requirement rations.

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TREATMENT OF CEREAL SEEDS BY DRY HEAT

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INTRODUCTION

In the investigation of possible control measures for certain seed-borne diseases of cereals which do not yield to the ordinary chemical and hot water seed treatments, the authors found dry heat to be particularly adaptable. The progress made with these seed treatments seems to warrant the publication of this preliminary paper, giving a brief review of the pertinent literature, as well as the methods employed and the results obtained by the writers to date.

REVIEW OF THE LITERATURE

The early literature, as well as some of the more recent papers relative to heat treatments, represents chiefly the results obtained by plant physiologists who were studying the effect of high temperatures and drying on germinability of various seeds, including those of certain cereals.

Edwards and Colin (*9*),[1] in 1834, were the first to make important contributions on the subject.

Heiden (*14, p. 30–37*), in 1859, showed that barley germinated after being exposed for one hour to dry air at 90° C., while similar grains heated in water at 60° C. for the same period of time were killed.

Sachs (*24*), in 1865, showed that moistened seeds of rye, barley, corn, peas, and flax were killed at from 50° to 60° C., while those containing less moisture withstood 70° C. Length of exposure was not mentioned.

Just (*17, 18*), in 1875 and 1877, found that clover and other kinds of seeds heated in a saturated atmosphere at 50° C. for 48 hours, or at 75° C. for 1 hour, lost their viability, while similar seeds endured a dry heat of 120° C. for 1 hour.

Von Höhnel (*15*), in 1877, working with the seeds of various plants, reported that most of them when dry were able to endure exposure to 110° C. for 60 minutes and that some were found viable even when exposed to 125° C. for 15 minutes.

[1] Reference is made by number (italic) to "Literature cited," p. 388–390.

Nobbe (*23*), in 1897, dried rye grain at 80° C. for several hours with very little effect upon subsequent germination. More severe exposure to dry heat was found to injure seriously the germinability of rye, while wheat and oats were killed at shorter exposures.

Jodin (*16*), 1899, reports that if seeds of peas and cress are dried at 60° C. for 24 hours they can endure dry heat at 98° C. for 6 hours without injury, while similar seeds heated in humid air at 40° C. for 20 hours lose their viability.

Dixon (*7*, *8*), in 1901 and 1903, found that if various kinds of seeds were previously dried over sulphuric acid in a desiccator they could withstand exposure to 95° C. for several days without losing their viability. He reports that well-dried seed will endure even somewhat higher temperatures, that is, 110° to 120° C., without injury.

Schneider-Orelli (*25*, *26*), 1909 and 1910, Neuberger (*22*), 1914, Waggoner (*29*), 1917, Harrington and Crocker (*13*), 1918, and others (*5*, *6*, *11*, *12*), working with various seeds, have confirmed, in general, the results of former investigators—that various seeds are able to endure high temperatures, especially when their moisture content is low.

It was not until about 1900, however, that plant pathologists began to appreciate the possibility of using dry heat as a means of control for certain seed-borne diseases.

In 1908 there appeared almost simultaneously several papers on the subject of smut control by dry heat. Kühle (*19*) reported that the spores of stinking smut are killed when exposed to 65° C. for 12 minutes and that loose smut also can be controlled by dry heat. For barley he used temperatures up to 90° C. and for wheat up to 110° C. without destroying the viability of the seed. The barley treated in this way was free from loose smut, but the results with wheat were not so successful. While Kühle does not mention the length of time during which the seed was treated, it probably is the same as that reported the same year by Störmer (*27*), who worked with him. Störmer used samples which were first dried at low temperatures and then heated for 10 minutes at each of the following temperatures: 50°, 65°, 75°, and 90° C. for barley and 50° to 60°, 65°, 85°, and 100° to 110° C. for wheat. The samples were then cooled down to room temperature after each 10-minute heat treatment. The results showed a marked decrease of smut in barley and only a slight decrease of smut in wheat.

For several years following 1908, dry heat treatments were abandoned by plant pathologists for a modified heat treatment in which seeds were first soaked in water and then subjected to high temperatures. This method was used with some success by Appel (*1*), Appel and Riehm (*2*, *3*, *4*), Gisevius and Böhmer (*10*), Lang (*20*), Störmer (*28*), and Westerdijk (*30*) for smut control. For example, Appel and Riehm (*2*, *3*) soaked smutted seeds of barley and wheat in water for 4 hours and then heated them at 55° to 60° C. for 20 to 30 minutes. These

exposures gave smut-free plants, but the 30-minute treatment greatly reduced the percentage of germination.

Naumov (*21, p. 149-162, 175*), in 1916, after having obtained negative results from all known seed treatments in attempts to control wheat scab (*Gibberella saubinetii* (Mont.) Sacc. and *Fusarium* spp.), reports that he was able to kill the infections on the seeds of cereals by dry heat. Wheat, barley, and oats were subjected to 60° C. and rye to 65° C. for periods ranging from 24 hours to 3 days. This treatment, according to Naumov, killed the fungus mycelium present in the interior of the kernels or at least weakened it greatly.

EXPERIMENTS

The writers first attempted to duplicate Naumov's treatments, and found his results difficult to verify. Wheat and barley thus treated retained their viability, but so did the fungi *Gibberella saubinetii* and the Fusarium, Helminthosporium, and Alternaria species infecting the kernels of these grains.

Following this, higher temperatures and longer exposures were tested with rather surprising results. Some wheat and barley kernels remained viable even after an exposure to 100° to 110° C. for as long as 45 hours. It was soon found possible by somewhat reducing this time to lessen the injury to the seed and yet kill the most persistent parasites. The barley used in these earlier experiments was Chevalier, a 2-row variety, abundantly infected with *Helminthosporium sativum* P. K. B. and also to some extent with *Gibberella saubinetii*. The kernels most badly infected with *H. sativum* are readily detected by the dark brown germ ends; hence, such kernels were selected for the experiments. This fungus has an added advantage for experimentation in that it sporulates freely on culture media and can be identified readily. Furthermore, *H. sativum* in the seed is more difficult to kill by the ordinary methods of seed disinfection than most other parasites known to the writers. On account of this resistance to seed treatments and because it is easily identified, *H. sativum* was chosen as the main index of efficiency of the dry-heat treatments tried. The wheat used in the earlier trials was a durum wheat, Kubanka (South Dakota 75), which was infected with Gibberella, Fusarium, Helminthosporium, and Alternaria.[1]

With the exception of one series these experiments were all made in a gas-heated sterilizing oven. While it required considerable attention throughout the duration of the treatments to keep the temperatures reasonably constant, this was accomplished by careful watching and the regulation of gas supply and ventilation as necessary.

[1] This was kindly furnished by Prof. Manley Champlin, of the South Dakota Agricultural Experiment Station, Brookings, S. Dak.

EXPERIMENT 1

In experiment 1, small lots of infected kernels of the Kubanka durum wheat and Chevalier barley were selected. These were exposed to 100° to 110° C. in the gas oven for 15-hour and 30-hour periods. About 10 series of culture experiments were made on these treated seeds to compare them with the untreated. In each culture 10 kernels each of barley and wheat were placed on potato agar poured plates and incubated at room temperatures. Surface disinfection with mercuric chlorid solution (1:1,000), for 30 minutes was used on the untreated kernels in order to disinfect the surfaces. In practically all cases Gibberella, Fusarium, Helminthosporium, and Alternaria developed rather uniformly from the unheated kernels of wheat and barley, as well as from the kernels which were heated for 15 hours. From the kernels heated for 30 hours, however, only one wheat kernel yielded the fungus Gibberella, and one barley kernel yielded Helminthosporium. In both of these cases the fungus growth was very weak and remained so.

EXPERIMENT 2

Following these promising leads various grains were treated in a large electrically heated drying oven at a temperature of about 100° C., in order to test the effect of 15-hour and 30-hour exposures on germinability. Following the treatment 100 kernels were counted out from each heated sample and the same number from its untreated control and sown in sand in the greenhouse. Table I gives the germination results for this experiment:

TABLE I.—*Effect of dry-heat treatment on germination of seed*

Kind of grain.	Variety.	Percentage of germination.		
		Not treated.	Exposed at about 100° C. for—	
			15 hours.	30 hours.
Barley	Hulless	94	72	71
Do	Beldi	73	69	47
Do	Manchuria (crop of 1917)	100	96	88
Do	Manchuria (crop of 1912)	96	79	61
Wheat	Winter wheat	93	92	91
Do	Turkey	96	80	78
Do	Kharkov	99	97	81
Do	Kanred	98	100	98
Do	Russian	100	99	97
Do	Marquis	100	93	94
Do	Dawson Golden Chaff	93	73	92
Do	Marquis	92	94	72
Rye	Winter rye	98	88	82
Oats	Wisconsin Pedigree No. 1	98	94	90
Do	Wisconsin Pedigree No. 5	95	66	62
Do	Sixty-day (South Dakota 165)	98	81	67
Do	Swedish select (South Dakota 112)	90	90	85

The results from this experiment show convincingly that good dry seed of barley, wheat, oats, and rye is able to withstand surprisingly well the high temperature used, up to 30 hours. Previous tests had shown this time and temperature to be fatal to even the persistent parasites. The Beldi barley used in the foregoing experiment was moderately infected with *Helminthosporium sativum*. Good data on the effect of the treatment on this parasite were obtained in the germination boxes as follows: Nine of the 73 plants from the untreated control seed and 9 of the 69 plants from the seed treated for 15 hours developed typical primary lesions of *H. sativum* and showed marked basal browning, while none of the 47 plants from the seed treated 30 hours showed either primary lesions of any kind or any basal browning. All plants from the treated seed were a trifle slow in starting, but in the second-leaf stage they had overtaken or surpassed the others and continued to develop normally until taken out. Table II summarizes the results from this barley infected with *H. sativum*.

TABLE II.—*Effect of dry-heat treatment in experiment 2 on germination and the development of Helminthosporium sativum in Beldi barley*[1]

Treatment.	Number of kernels sown.	Number of kernels germinated.	Number of plants with primary leaf lesions.	Basal browning.
None	100	73	9	+
15 hours at 100° C.	100	69	9	+
30 hours at 100° C.	100	47	0	−

[1] Seed sown in sand Mar. 28. Observations made Apr. 9.

While this was a weak sample of barley, as shown by percentage of germination in untreated seed, the results as to the effect of the treatment on the disease are striking—the 30-hour treatment completely eliminated the disease.

EXPERIMENT 3

Two other series of treatments were then undertaken, and the seed treated was used for further germination and infection experiments and in field sowings. In these experiments only seeds that were known to be infected with various diseases were used. The third experiment was started April 22 and completed April 23. The gas oven was used, as in experiment 1, and was watched very carefully through the 30 hours. Observations of the temperature were made at least every half hour and during most of the time at shorter intervals. The temperature range was from 95° to 105° C., averaging about 100° throughout. Samples of about 1 pint each of four different seed lots were used for germination tests and field sowings. For the germination tests, 100 kernels were counted from each treated sample and a like number from each corresponding

untreated control. They were all sown in a thin layer of sterile sand over sterile garden soil in the greenhouse. Table III summarizes the results obtained.

TABLE III.—*Effect of dry-heat treatment in experiment 3 on seed germination and the development of Helminthosporiun sativum in barley*

Kind of grain.	Variety.	Number of kernels germinated.		Infection.			
				Untreated.		Treated.	
		Untreated.	Heated at 100° C. for 30 hours.	Number of plants with primary leaf lesions.	Basal browning	Number of plants with primary leaf lesions.	Basal browning.
Barley.....	Chevalier.....	85	71	21	+	0	—
Do.....	Oberbrucker.....	94	85	5	+	0	—
Do.....	Manchuria.....	97	58	1	+	0	—
Do.....	Beldi.....	86	55	14	+	0	—

Table III shows two things: First, that the barley was not killed by the very severe treatment, in fact proved quite resistant; second, that while there were heavy infections of *Helminthosporium sativum* in the untreated seed lots, there was perfect control of the disease in those treated. These conditions are illustrated in general in Plates 48 and 49.

In Plate 48, A, the two groups represent all the 85 plants resulting from the greenhouse experiment on the 100 untreated kernels of Chevalier barley referred to in Table III. At the left are shown the 21 plants with distinct leaf lesions from attacks of *Helminthosporium sativum*, while in the larger group at the right are represented the remaining 64 plants from the untreated seed that did not show leaf lesions. That all the plants in both groups showed marked darkening of the kernels is evident, also that many showed markedly discolored roots. The dark color of these kernels and the root discolorations are shown more strikingly in Plate 49, A, on the 5 plants at left.

In Plate 48, B, are shown all the 71 plants resulting from parallel greenhouse experiments on the 100 treated seeds of Chevalier barley referred to in Table III. These were perfectly free from *Helminthosporium sativum* attacks. Their bases were usually clear and clean. The kernels were much lighter-colored than untreated ones (Pl. 48, A), and the roots also were free from discoloration. This is more clearly seen in Plate 49, A, where typical plants from both groups are represented. The 5 plants at the right are from treated seed, while the 5 at the left are from untreated seed.

Although the infections were less severe in the other varieties, the results agree in general with those brought out in detail for the Chevalier barley. That is, infections resulted from untreated seed, and perfectly clean plants resulted from dry-heat-treated seed as shown in Table III.

EXPERIMENT 4

The fourth experiment was started April 25 and completed April 26, 1918, the seed being heated for 30 hours. The same gas oven was used as for the third experiment. It was watched in the same way and the temperature range held the same, 95° to 105° C., averaging about 100°. Seed samples of about 1 pint were again used, but in this fourth experiment wheat, oats, and rye were tested.

In Table IV are given the results of greenhouse germination tests on the untreated and treated seed of both the third and fourth experiments.

TABLE IV.—*Effect of dry-heat treatment in experiments 3 and 4 on germination*

Kind of grain.	Variety.	Percentage of germination.	
		Untreated.	Heated for 30 hours at 100° C.
Barley	Beldi	86	55
Do	Beldi[1]	53	63
Do	Chevalier	85	71
Do	Oderbrucker (a)[2]	95	64
Do	Oderbrucker (b)	94	79
Do	Oderbrucker (c)	100	31
Do	Oderbrucker (d)	92	59
Do	Oderbrucker (e)	95	66
Do	Oderbrucker (f)	95	85
Do	Manchuria	97	58
Do	Manchuria (Wisconsin pedigree No. 9)	97	83
Wheat	Kubanka (South Dakota No. 75)	66	30
Do	Preston (South Dakota No. 67)	95	53
Do	Kanred	100	98
Do	Kharkov	97	80
Rye	Winter rye	92	16
Oats	Mixed variety	96	65
Do	Swedish Select	95	57

[1] Seed hand-picked from plants distinctly infected with bacterial blight in the heads.

[2] Oderbrucker a, b, c, etc., represent samples of same variety but from different sources.

It is evident from Table IV, as from Table I, that seeds of these various cereals withstand this severe drying surprisingly well. While with certain samples the germination was cut down severely, as with rye and Preston wheat, it should be noted that both of these were rather shrunken, especially the Preston wheat. The Kubanka wheat was also a weak sample.

The seed of the wheat varieties, Kubanka (South Dakota No. 75) and Preston (South Dakota No. 67), used in experiment 4, carried a considerable amount of scab infection (*Gibberella saubinetii* and *Fusarium* spp.). The untreated grain from both samples when sown in the greenhouse for the germination test gave a considerable number of plants showing seedling infections. The bases of many plants were discolored, and some plants were killed even before reaching the surface (Pl. 49, B, at left).

These symptoms are typical of seedling infections from the scab organism. The seedlings from the wheat of both varieties treated by the dry-heat method were free from any indication of disease (Pl. 49, B, at right).

FIELD SOWINGS AND RESULTS

Field sowings were made of all the seed lots of the barley, wheat, rye, and oats treated in experiments 3 and 4, as listed in Table IV. The sowings were made in an isolated place on the university farm at Madison, Wis. Seed from each lot was sown in from one to five rows, each 150 feet long and about 12 inches apart. Care was taken throughout to avoid contamination of seed from any source. In order to prevent secondary infections from other fields no similar grains, not even the control seedlings, were grown within about ¾ mile of this plot. The control seedings of a complete parallel series of untreated seed lots were grown in another field of similar soil type, elevation, and exposure. The plants, both from the treated and untreated seed lots, developed normally; and good stands resulted except from the Preston and Kubanka wheats. These stands were thin.

While the results with regard to disease on plants from the dry-heat-treated seeds were in certain respects disappointing, yet in others they were rather encouraging, and in still others they were very satisfactory, as shown by the following brief account.

BACTERIAL BLIGHT OF BARLEY.—The bacterial blight of barley (*Bacterium translucens*, J. J. R.) was controlled perfectly by the dry-heat treatment as used, not even the slightest trace of this disease being noted in the plot from the treated seed, though the seed was known to be heavily infected. The corresponding plot from untreated seed, on the other hand, showed abundant infections with the disease.

The perfect control of the bacterial disease of barley is highly significant. The results were very definite and striking—perfect control in the treated plots and abundant disease in the untreated plots. Furthermore, there are indications that this bacterial organism of barley is more resistant in the seed than is that of bacterial disease of wheat known as "blackchaff." The above results would indicate the very strong likelihood that this dry-heat treatment will prove highly efficient in controlling the "blackchaff" of wheat. The data given above show definitely that wheat of good quality will stand the treatment.

BACTERIAL BLIGHT OF OATS.—The perfect control of the bacterial blight of oats (*Pseudomonas avenae*) was equally definite. It was the more striking because this disease was general throughout southern Wisconsin during the season of the experiment (1918). In fact, while numerous fields were examined, the only field of oats noted where this bacterial disease could not be found was the plot sown from dry-heat-treated seed. The untreated plot showed abundant infection with the

disease. Oats in good condition will withstand successfully the 30-hour treatment, but it is probable that even a less severe exposure will be found to control the organism effectively.

WHEATSCAB.—The Kubanka and Preston wheats used in experiment 4 were heavily infected with scab (*Gibberella saubinetii* and *Fusarium* spp.). The seedlings from the treated seed showed no attacks from this disease, while the ones in the control plot from the untreated seed showed numerous primary infections. Likewise later, when in head, the plants from the treated seed showed no scab in the head, while the wheat in the isolated control plot showed an abundance of such infections. These data, while yielding encouraging indications, of course point only to the possibility of eliminating seed infection.

SPOTBLOTCH OF BARLEY.—The Chevalier barley used in experiment 3 was heavily infected with spotblotch (*Helminthosporium sativum*), as illustrated in Plate 48, A. The thousands of plants in the plot from treated seed were carefully examined, and only four leaf lesions were noticed on the young seedlings. This would seem to indicate that spotblotch was not quite perfectly eliminated in the field, though from the greenhouse experiments previously mentioned its elimination might have been expected. The disease was present in considerable abundance in the control plot planted with untreated seed.

NETBLOTCH AND STRIPE DISEASE OF BARLEY.—Scatteriug primary infections of netblotch (*Helminthosporium teres*) on barley and several infections of stripe disease of barley (*H. gramineum*) were noted in the plots planted with the dry-heat-treated seed. An abundance of both these diseases occurred in the control plot planted with the untreated seed. The same was true of the Helminthosporium leafblotch of oats (*H. avenae-sativae*).

SMUT.—The percentage of loose smut infection in barley and oats was considerably diminished by the 30-hour heat treatment. In comparison with the numerous smutted heads in the control plots only a few appeared in the plots sown with treated seed.

Work is being continued on the problem.

SUMMARY

(1) The work here reported, while only a beginning, suggests promising possibilities.

(2) The data at hand indicate that the various cereals—barley, wheat, rye, and oats—especially when of good quality and well-dried, are able to withstand protracted exposures to dry heat at comparatively high temperatures.

(3) It is definitely shown that the seed infections from bacterial blight of barley (*Bacterium translucens*) and the bacterial blight of oats (*Pseudonomas avenae*) may both be eliminated by exposing the infected seed to dry heat at temperatures which leave the seed still viable.

(4) The results of these experiments indicate that a number of seed-borne fungous diseases, such as wheatscab (*Gibberella saubinetii* and *Fusarium* spp.), primary infections only, and spotblotch of barley (*Helminthosporium sativum*), are practically eliminated by the dry-heat treatment as used. Other diseases like netblotch (*H. teres*), stripe disease (*H. gramineum*) of barley, and Helminthosporium blotch of oats (*H. avenae-sativae*), as well as loose smut of barley and smuts of oats, are markedly reduced by the dry-heat treatment without materially injuring the germination of the seed.

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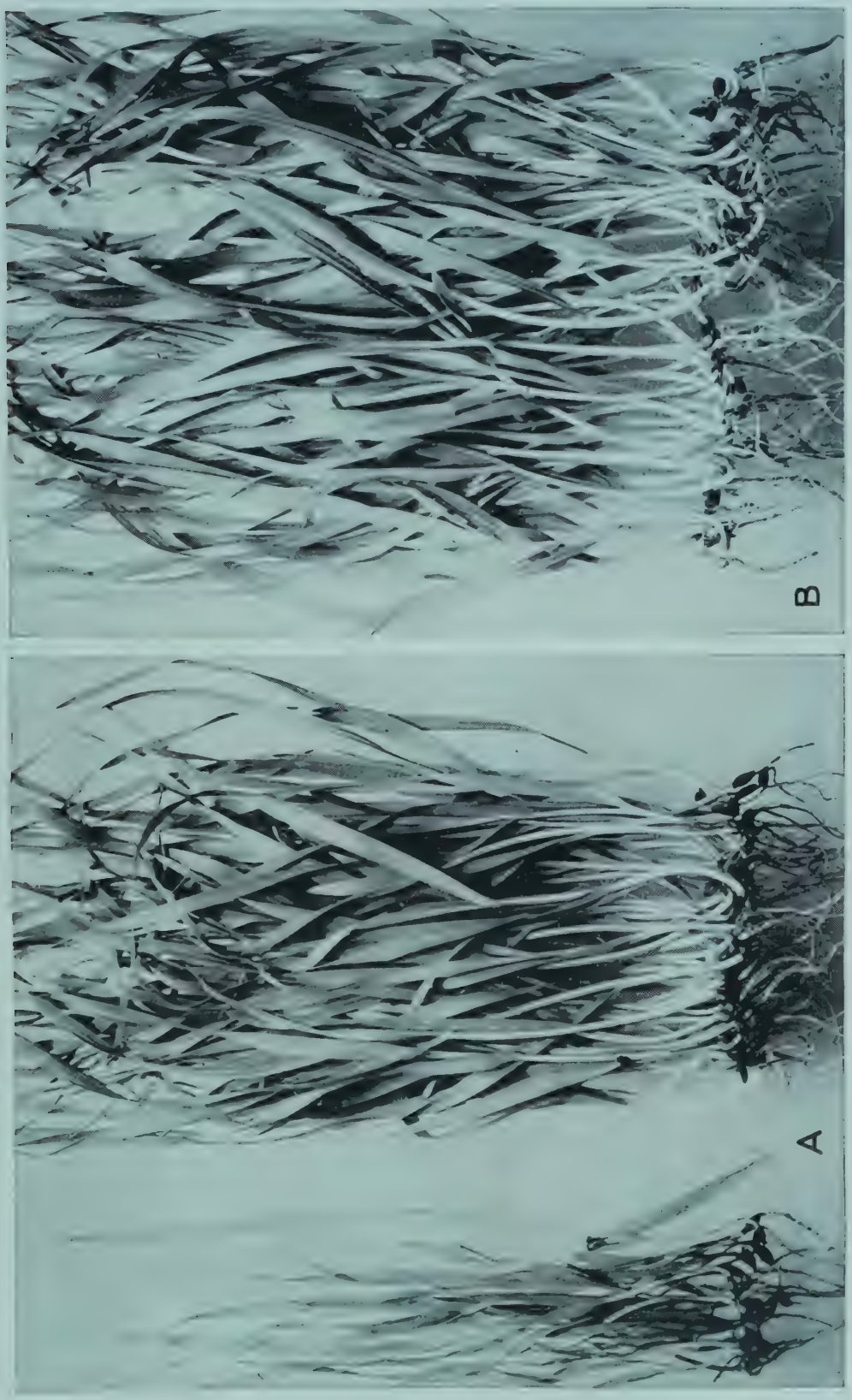
PLATE 48

Chevalier barley plants from untreated and treated seed referred to in Table III:

A.—Plants from untreated seed. At left are illustrated the 21 plants showing the most marked evidence of *Helminthosporium sativum* primary infection: leaf lesions, basal browning, and root rotting. At right are illustrated the other 64 plants showing less marked evidence of infection. Roots and kernels, however, show considerable discoloration.

B.—Plants from seed treated with dry heat. No diseased plants whatever; all 71 plants are clean and roots bright.

Approximately one-third natural size.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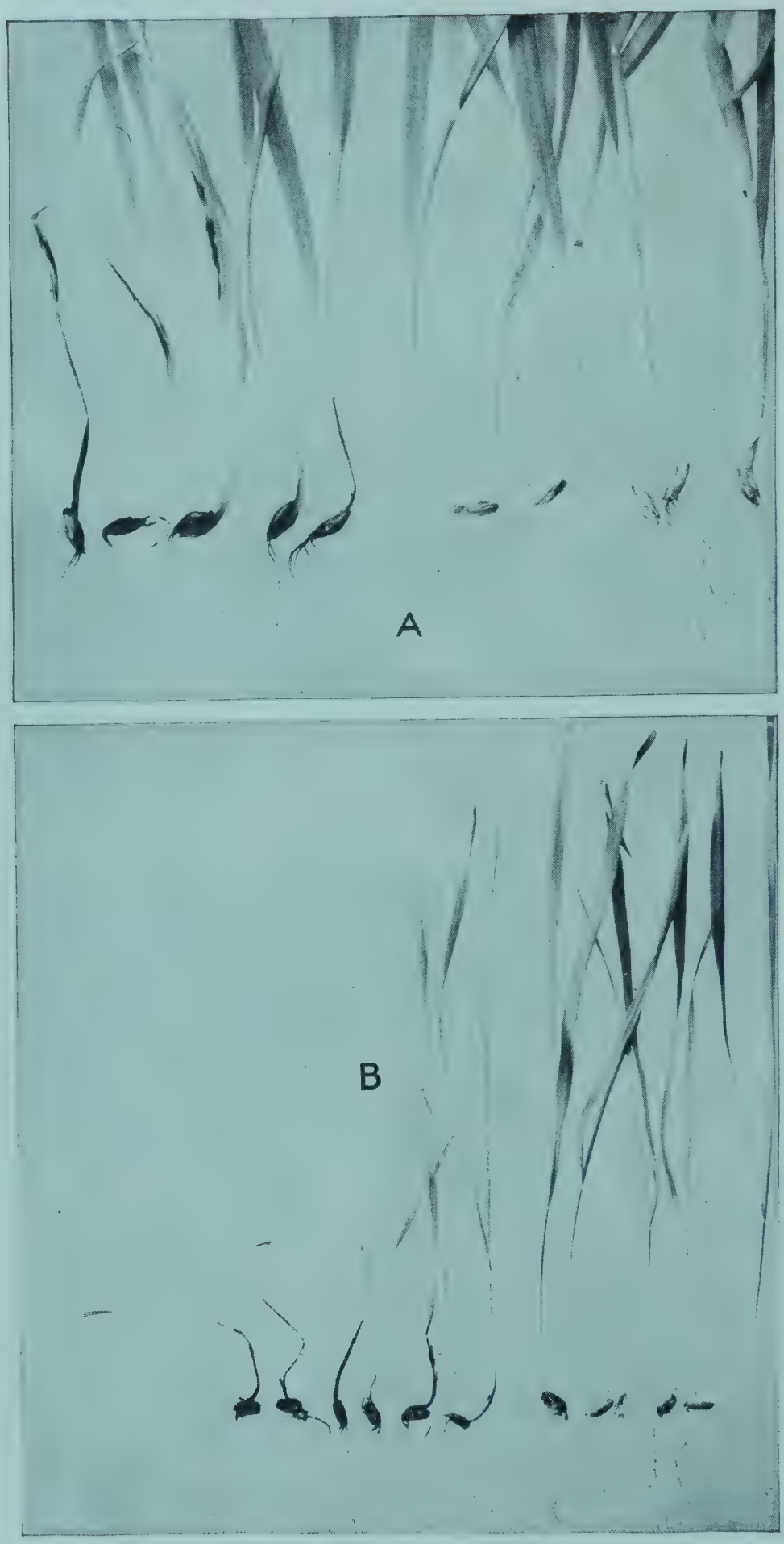
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PLATE 49

A.—Basal portions of 10 representative Chevalier barley plants, from untreated and treated seed, selected from the lots illustrated in Plate 48. The 5 plants at left are from those shown in Plate 48, A, from untreated seed. Note the marked *Helminthosporium sativum* leaf lesions, basal browning, kernel discoloration, and root rotting. The 5 plants at right are from those shown in Plate 48, B, from the treated seed. Note the freedom from disease. There is no evidence of leaf lesions, basal browning, kernel discoloration, or root rotting; roots are clean and bright. All plants from seed treated by the dry-heat treatment were a trifle slower in germinating, but in the second leaf stage they had overtaken or surpassed those from untreated seed. This sturdier growth is evident in the illustration.

B.—Representative Kubanka wheat seedlings from untreated seed (6 plants at left) and treated seed (5 plants at right) referred to on pp. 385–386. The 6 plants at the left show characteristic seedling injury from wheatscab organisms. All 6 of these plants had discolored kernels, rotted bases, and rotted proximal portions of roots. They were also much weaker than those from the treated seed. The first 2 plants on the left were killed after they reached the surface of the ground; the third plant was killed before reaching the surface of the ground. Typical *Gibberella perithecia* developed on this dead plant under the soil and at the surface. The 5 plants at the right represent the disease-free condition of plants from treated seed from the same seed lot. This seed had been exposed to dry heat at about 100° C. for 30 hours. The plants are free from any evidence of disease, the bases, kernels, and roots all being clean. Plants from treated seed are also much sturdier than those from untreated seed.

Approximately one-half natural size.

MEAT SCRAPS VERSUS SOYBEAN PROTEINS AS A SUPPLEMENT TO CORN FOR GROWING CHICKS

By A. G. PHILIPS, *Chief in Poultry Husbandry*, R. H. CARR, *Associate in Nutrition*, and D. C. KENNARD, *Assistant in Poultry Husbandry, Purdue University Agricultural Experiment Station*

The proteins found in natural feed stuffs vary greatly in their nutritive value as well as in their solubility and in the proportions of the different amino acids which they are capable of yielding. The cereal grains contain most of the important amino acids but apparently in many cases in proportions unsuitable to promote growth and development. McCollum and his coworkers [1] have shown that the cereal grains, although they have a low biological value as compared to milk, have a remarkable value as supplementary sources of amino acids for certain vegetable proteins. It is thought that the value of corn proteins in producing growth has been somewhat underestimated. This may be due to the fact that one of the proteins (zein) which is usually present in a considerable amount, lacks two important amino acids (lysin and tryptophane), and the young animal fed on corn is incapable of appreciable growth on the only proteins remaining (glutelin, globulins, and albumins). Data obtained by R. H. Carr and coworkers [2] would seem to indicate that the amount of zein in mature corn is somewhat overestimated, and hence that the other proteins present were probably largely responsible for the consistent growth which was secured with chicks fed on corn containing less than 10 per cent proteins fortified with ash and with fat-soluble vitamines. The proteins of meats are credited with having a high value in producing growth, but their relative efficiency as compared with vegetable protein is not so well understood. The proteins of soybean are usually considered of excellent quality, [3] but their biological value is thought to be of the same order as that of corn and oats.

Most of the work done so far in measuring the biological value of proteins from various sources has been conducted on the rat or the pig, because these animals have many points of advantage for laboratory investigations. The growing chick has been used by some investigators,

[1] MCCOLLUM, E. V., SIMMONDS, N. and PARSONS, H. T. SUPPLEMENTARY RELATIONSHIPS BETWEEN THE PROTEINS OF CERTAIN SEEDS. *In* Jour. Biol. Chem., v. 37, no. 1, p. 155-178, 7 charts. 1918. Bibliography, p. 177-178.

[2] SPITZER, George, CARR, R. H., and EPPLE, W. F. SOFT CORN—ITS CHEMICAL COMPOSITION AND NITROGEN DISTRIBUTION. *In* Jour. Amer. Chem. Soc., v. 41, no. 8, p. 1212-1221. 1919.

[3] DANIELS, Amy L., and NICHOLS, Nell B. THE NUTRITIVE VALUE OF THE SOYBEAN. *In* Jour. Biol. Chem., v. 32, no. 1, p. 91-102. 1917.

notably Drummond,[1] who reports much difficulty in securing growth of the chicks in confinement. The chicks were troubled with "leg weakness" and "ruffled appearance," both of which defects were attributed to the lack of exercise in the open air. Osborne and Mendel [2] report partial success in raising chickens in confinement. Although they also report much difficulty with the chickens on account of "leg weakness," they were successful in raising several birds which seemed to develop quite normally. There are good reasons why it is desirable to use growing chicks in the laboratory to test the biological value of feeds. They represent an entirely different species from that of the rat or the pig, and it is hardly logical to translate the results secured on those animals to a species lower than mammals in the evolutionary scale. The ease with which it is possible to hatch eggs in an incubator and the rapid rate at which chicks grow and reach maturity, as well as the comparatively

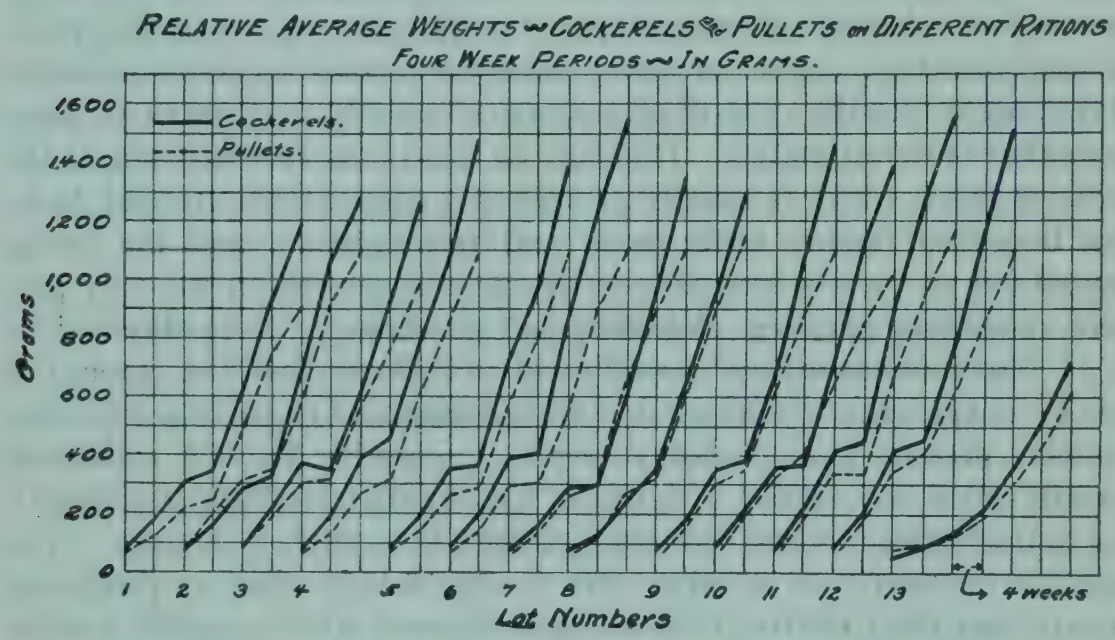


FIG. 1.—Graph showing the rate of growth of males and females in all lots.

small amount of feed required, are points in their favor for investigative work. The problem of securing normal growth in confinement has presented the greatest drawback to the successful use of chicks for this purpose. In the work here reported the writers had little difficulty with the disease known as "leg weakness" in the chicks, or with the other troubles usually experienced when rearing chicks in confinement, as reported in the literature. However, serious trouble was experienced from the eighth to twelfth week, when the chicks were developing a heavy growth of plumage. During this time 64 per cent of the total mortality occurred. The immediate cause of mortality was apparently excessive intestinal fermentation. After this critical stage was passed no further

[1] DRUMMOND, Jack Cecil. OBSERVATIONS UPON THE GROWTH OF YOUNG CHICKENS UNDER LABORATORY CONDITIONS. *In* Biochem. Jour., v. 10, no. 1, p. 77–88, 1 pl. 1916.

[2] OSBORNE, Thomas B., and MENDEL, Lafayette B. THE GROWTH OF CHICKENS IN CONFINEMENT. *In* Jour. Biol. Chem., v. 33, no. 3, p. 433–438, pl. 4–6. 1918.

trouble was experienced, and practically all the remaining chicks developed in a normal way. See figure 1.

OBJECT OF EXPERIMENT

The object of this experiment was to determine the value of corn protein in the growth of chicks when the proteins were fortified with sufficient ash and with fat-soluble vitamines, as compared with their value when supplemented by varying amounts of proteins derived from meat scraps or soybean meal or from these proteins in combination.

PLAN OF PROCEDURE

The stock used was 210-day-old White Leghorn chicks from the Purdue Poultry Farm. The chicks were hatched May 6 and divided into 14 lots of 15 chicks each. The initial individual weights of all the chicks were recorded on the sixth day, when all lots were given their respective rations tabulated in Table I. During the first 6 days all lots were given only granulated corn, grit, and water. Individual weights of the chicks were taken every 14 days after the initial weights were recorded. Each ration was "weighed back" on the same day the chicks were weighed in order to obtain the feed consumption for each period of 14 days. The growth period of the experiment was closed at the end of 26 weeks, but the pullets were kept for a longer time to note results of egg production.

The method of care and management of the chicks was that which is generally advocated for the successful rearing of chicks. Since the chicks were confined in pens 4 by 6 feet during the entire experiment, special effort was made to feed them so that they would be active as much of the time as possible and thereby avoid the evils of overfeeding.

Table I gives the rations, in part by weight, received by each lot.

In addition to these rations, each lot received water, grit, oyster shells, and about 75 gm. of the tops of sprouted oats. Oat straw was used for scratching litter. Lot 13, used as a control pen, received the basal ration only. The ash mixture was omitted from the ration of lot 2a as a control on the ash; otherwise this lot received the same ration as lot 2. Since there was no appreciable difference in the results obtained from these two lots, no further reference will be made to lot 2a. In each case the amount of protein concentrate (meat scraps or soybean meal) added to the basal ration is based upon a definite amount of crude protein from that source as shown in Table I. The amount of protein concentrate used depended upon its content of crude protein as determined by chemical analysis. Chemical analyses were made also of the other feeds which entered into the rations. The same feeds were used during the entire experiment.

TABLE I.—*Ration supplied to growing chicks during 26 weeks of experiment*

[Expressed in parts by weight]

Lot No.	Basal ration.						Meat scraps.	Soy-bean meal.	Nutritive ratio.
	Grain.		Mash.						
	Ground corn.	Corn meal.	Corn bran.	Ash.[a]	Charcoal.	Crude protein.			
1	50	35	15	3	3	5	8.86	.....	1:5.9
2	50	35	15	3	3	10	17.7	.....	1:4.4
2a	50	35	15	0	3	10	17.7	.....	1:4.4
3	50	35	15	3	3	15	26.6	.....	1:3.5
4	50	35	15	3	3	20	25.4	.....	1:2.9
5	50	35	15	3	3	5	.....	10.9	1:6.2
6	50	35	15	3	3	10	.....	21.8	1:4.8
7	50	35	15	3	3	15	.....	32.7	1:3.9
8	50	35	15	3	3	20	.....	43.6	1:3.4
9	50	35	15	3	3	5	4.4	54.5	1:6
10	50	35	15	3	3	10	8.86	10.9	1:4.5
11	50	35	15	3	3	15	13.3	16.4	1:3.7
12	50	35	15	3	3	20	17.7	21.8	1:3.2
13	50	35	15	3	3	.....	.....	.....	1:9

[a] The ash mixture used in the above rations was composed of the following ingredients, expressed in parts:

Bone ash	50
Calcium carbonate	14
Sodium chlorid	15
Dipotassium phosphate	10
Calcium lactate	5
Magnesium sulphate	3
Sulphur	2
Iron sulphate	1
	100

Table II shows the average total amount of feed and its protein content which was consumed in 13 periods of 14 days each and the ratio of the protein fed to the gain.

TABLE II.—*Ratio between average feed consumed and average gain in weight in 13 periods of 14 days each*

BASAL RATION PLUS MEAT SCRAPS

Lot No.	Feed consumed.	Protein in feed.	Protein consumed.	Average gain per 14-day period.	Ratio of protein in feed to gain.
	Gm.	*Per cent.*	*Gm.*	*Gm.*	
1	535	12.44	66.34	87.00	1:1.31
2	598	15.88	94.96	85.3	1:0.89
3	509	18.60	94.67	80.60	1:0.85
4	546	20.9	114.10	87.20	1:0.76
Average	547	.....	92.52	84.5	1:0.95

TABLE II. *Ratio between average feed consumed and average gain in weight in 13 periods of 14 days each*—Continued

BASAL RATION PLUS SOYBEAN MEAL

Lot. No.	Feed consumed.	Protein in feed.	Protein consumed.	Average gain per 14-day period.	Ratio of protein in feed to gain.
	Gm.	*Per cent.*	*Gm.*	*Gm.*	
5	530	12. 53	66. 40	89. 80	1:1. 35
6	631	15. 36	96. 92	104. 30	1:1. 08
7	533	17. 7	94. 34	97. 30	1:1. 03
8	551	19. 8	109. 10	90. 2	1:1. 83
Average	561	.....	91. 69	95. 30	1:1. 07

BASAL RATIO PLUS COMBINATION OF MEAT SCRAPS AND SOYBEAN MEAL

9	523	12. 64	66. 10	90. 9	1:1. 05
10	615	15. 6	95. 94	101. 20	1:1. 37
11	629	18. 11	113. 85	99. 80	0. 88
12	582	20. 31	118. 15	98. 00	1:0. 83
Average	587	.....	94. 60	97. 48	1:1. 03

BASAL RATIO ONLY

13	365	9. 12	33. 29	47. 92	1:1. 44

In this table the growth-promoting value of the protein is expressed numerically as suggested by Osborne and Mendel.[1] The table shows also that protein from meat scraps alone as a supplement to the basal ration in any amount was not equal to that from soybeans or from the combination of the two. Among the different lots (except the control lot 13) the average amount of feed consumed during the 13 periods of 14 days each did not vary greatly, ranging from 509 gm. for lot 2 to 631 gm. for lot 6. Hence it is quite important, from the standpoint of economy in feeding, to combine the amount and kind of protein with the basal ratio in the proportion which produces the best growth. In this instance the best results were shown by lot 6, which received 10 parts of protein from soybean meal. Next in order are lots 10, 11, and 12, which received 10, 15, and 20 parts protein, equally from meat scraps and soybean meal. Following these are the lots receiving protein from meat scraps. Plate 50 shows a cockerel and a pullet from lot 13, a cockerel from lot 10, and a pullet from lot 6.

[1] OSBORNE, Thomas B., MENDEL, Lafayette B., and FERRY, Edna L. A METHOD OF EXPRESSING NUMERICALLY THE GROWTH-PROMOTING VALUE OF PROTEINS. *In* Jour. Biol. Chem., v. 37, no. 2, p. 223-229. 1918.

The aim was to supply a sufficient amount of ash to each ration to meet all mineral requirements and so have but one variable protein running through the whole series of rations. The addition of ash to the rations containing meat scraps was probably unnecessary, but in order to secure uniformity the same amount was added to all.

Table III gives the percentage of nitrogen and ash in the feces at different periods of the experiment.

TABLE III.—*Distribution of feces nitrogen and ash at different periods*

Lot No.	Nitrogen and ash content of feces from chicks 4 weeks old.				Nitrogen and ash content of feces from chicks 20 weeks old.				Average total nitrogen in feces.[a]
	Total nitrogen.	Total nitrogen soluble in *N*/10 hydrochloric acid.	Total nitrogen insoluble in *N*/10 hydrochloric acid.	Ash.	Total nitrogen.	Total nitrogen soluble in *N*/10 hydrochloric acid.	Total nitrogen insoluble in *N*/10 hydrochloric acid.	Ash.	
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
BASAL RATIO PLUS MEAT SCRAPS									
1	2.63	35.0	65.0	31.04	3.25	37.6	62.2	24.69	2.73
2	3.63	35.8	64.2	35.91	3.76	29.1	70.9	27.76	3.56
3	2.76	31.8	68.2	40.18	3.45	18.5	81.5	27.26	3.35
4	4.21	35.7	64.3	32.99	6.23	15.7	84.3	20.83	4.44
Average	3.31	.....	.....	.....	4.17	.....	.....	.....	3.52
BASAL RATIO PLUS SOYBEAN MEAL									
5	2.46	35.1	64.9	28.94	3.33	25.2	76.8	18.44	2.74
6	2.72	35.9	64.1	28.49	4.59	19.1	80.9	20.62	3.62
7	3.05	26.4	73.6	31.49	5.61	16.9	83.1	17.77	4.49
8	2.63	25.1	74.9	44.55	7.23	10.8	89.2	19.00	4.42
Average	2.71	.....	.....	.....	5.19	.....	.....	.....	3.82
BASAL RATIO PLUS COMBINATION OF MEAT SCRAPS AND SOYBEAN MEAL									
9	2.68	31.0	69.0	25.07	3.95	20.6	79.4	17.12	2.91
10	2.59	37.1	61.9	32.89	4.87	18.1	81.9	28.59	3.52
11	3.02	31.6	68.4	36.84	5.45	24.5	75.5	17.72	4.00
12	4.00	32.5	67.5	36.30	7.63	14.6	85.4	22.26	4.92
Average	3.07	.....	.....	.....	5.48	.....	.....	.....	3.84
BASAL RATIO ONLY									
13	1.91	34.4	65.6	31.75	2.70	26.9	73.1	15.36	2.24

[a] Average total nitrogen is the average for the 4-, 8-, 16-, and 20-week periods.

The table may be summarized as follows:

Lot No.	Ration.	Average total feces nitrogen.	Increase in waste.
		Per cent.	*Per cent.*
13	Basal	2.24	.....
1, 5, 9	Basal+5 parts protein	2.79	24.5
2, 6, 10	Basal+10 parts protein	3.57	59.4
3, 7, 11	Basal+15 parts protein	3.95	76.3
4, 8, 12	Basal+20 parts protein	4.59	105.0

The nitrogen soluble in *N/10* hydrochloric acid was considered to be ammonia, urea, and amino acid nitrogen. The nitrogen insoluble in N/*10* hydrochloric acid was considered to be uric acid and residual nitrogen.

The data in Table III indicate that in all lots receiving 5 parts of protein in addition to the basal ration, the excreta contained an average of 2.79 per cent nitrogen; in lots receiving 10 parts protein, they contained an average of 3.57 per cent; in lots receiving 15 parts, they contained an average of 3.95 per cent; in lots receiving 20 parts protein, they contained an average of 4.59 per cent; whereas in the lot receiving the basal ration only, they contained an average of 2.24 per cent nitrogen. This last figure was taken as maintenance nitrogen excretion. Since the feces in lots receiving 5 parts protein in addition to maintenance contained 2.79 per cent nitrogen, it was computed that the waste in excretion was 24.5 per cent greater than when the basal ration alone was fed. In the same manner 59.4 per cent more feces nitrogen was obtained for lots receiving 10 parts protein, 76.3 per cent more for lots receiving 15 parts, and 105 per cent more for lots receiving 20 parts. In brief, the greatest gain in weight was made with the least necessary nitrogen loss in feces when the basal ration was supplemented with 10 parts of protein.

It will be noted in Table III that the ash content of the feces collected when chicks were 4 weeks old and growth was most rapid was much greater for all lots than that of the samples collected when the chicks were 20 weeks old and growth was less rapid and maintenance requirements were greater. The excretion of nitrogen was very constant for all lots receiving the same amount of protein; and since the protein consumed increased by 5 parts in four successive rations, the average increases over control lot 13 were 2.79, 3.57, 3.95, and 4.59 per cent, respectively, for each addition of 5 parts of protein to the basal ration. Thus it would appear that there was no economy in nitrogen excretion at the point where the gain was most efficient (the addition of 10 parts protein), though such an economy might have been expected. Table III also shows that a 2-gm. sample of feces of any nitrogen content contained nearly the same weight of *N/10* acid-soluble nitrogen (ammonia,

urea, and amino acids) regardless of the total weight of nitrogen in the sample, and that only the uric acid, etc., increased in amount as more protein was fed.

SUMMARY

In conclusion, it would seem that it is possible to secure nearly normal growth of chicks when raising them in confinement, and that this method has many points of advantage as a means of measuring the biological value of feeds for chickens.

These results indicate that there is a wide range in the amount of protein which may be fed with little difference in results except in economy in feed consumption.

When the basal ration was supplemented with varying amounts of protein from meat scraps, soybean meal, or combination of the two, it is shown that an addition of 10 parts of protein from soybean meal gave the best growth. The next best gains came from 10, 15, and 20 parts of protein from the combination of soybean meal and meat scraps. All the meat scraps rations were found to be somewhat inferior to those of the soybean meal or the combination.

The amount of nitrogen present in the feces as ammonia, urea, or amino acids (soluble in *N/10* hydrochloric acid) was nearly constant regardless of the total nitrogen present in any sample, the remainder of the nitrogen present being due largely to the uric acid. The amount of excreted nitrogen was dependent on the amount of the protein consumed and increased proportionately.

The data which have been presented tend to show that chicks are capable of greater utilization of soybean meal protein than are mammals, with which nearly all previous nutritional work has been done.

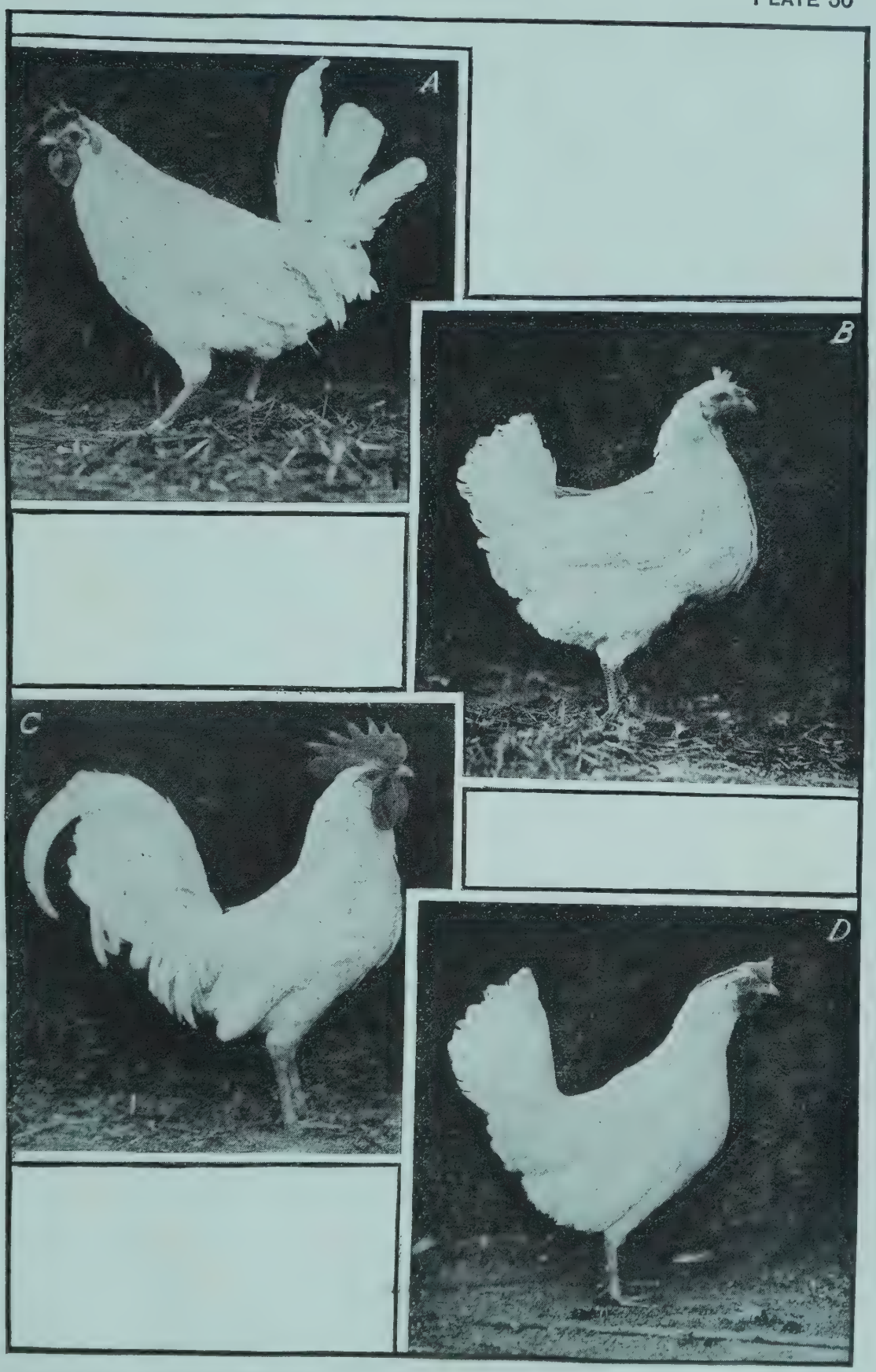
PLATE 50

A.—Cockerel No. 43, lot 13, fed on basal ration only. Weight, 710 gm. In perfect health but slow of growth.

B.—Pullet No. 44, lot 13, fed on basal ration only. Weight, 705 gm. Feathers not mature. Bird growing slowly but naturally, and gradually approaching maturity.

C.—Cockerel No. 54, lot 10, fed on basal ration plus five parts meat scraps protein and 5 parts soybean meal protein. Weight, 1,620 gm. Bird vigorous and normal in every respect.

D.—Pullet No. 82, lot 6, fed on basal ration plus 10 parts soybean meal protein. Weight, 1,295 gm. Bird vigorous, laying, and normal in every way.


A
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D

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FURTHER STUDIES OF SOROSPORELLA UVELLA, A FUNGOUS PARASITE OF NOCTUID LARVÆ

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INTRODUCTION

Because of their peculiar mode of life and varied characters, the Fungi Entomogeni, or fungous parasites of insects, possess an unusual interest and have formed the subject of a number of classic papers in mycological literature. Not only are they of interest from a scientific point of view, but they possess an economic importance which is probably much greater than is generally supposed.

While the majority of mycologists are likely to overlook these fungi through a lack of familiarity with insects and their habits, it is equally true that an ignorance of mycology has usually led entomologists to give them at best but scant attention, even when their presence is evident. That they are often overlooked is due further to the fact that even in destructive forms their development usually takes place within the living insect. The externally visible growth which occurs, if at all, after death is usually more or less concealed because of the position of the host at this period; and in many instances it may be so evanescent that after a relatively short interval the cause of death can not be determined.

The fungi of this nature which are of economic importance belong for the most part to one of two categories, the Entomophthorales and Ascomycetales, while associated with the latter may be distinguished a third group of entomogenous "Fungi Imperfecti," which although assumed for the most part to be imperfect stages of ascomycetous forms, have not been definitely connected as yet with any perfect or acigerous condition.

In all the groups the economic value of the parasites concerned is due to the fact that their cycle of development may be a very short one and that in all cases an enormous provision is made for reproduction, a provision vastly greater than that of any of the many types of parasitic insects, an increasing number of which are now being used in an effort to control noxious forms.

[1] The writer wishes to express his appreciation to Dr. Roland Thaxter, of Harvard University, for helpful criticism of the work while it was in progress, and to Mr. W. H. White, of the Bureau of Entomology, United States Department of Agriculture, who first found the parasite.

The artificial propagation and dissemination of such fungous diseases in cases where they may be readily controlled is thus, evidently, a matter of no slight importance; and the necessity for a complete and exact knowledge of their development and life cycle in all cases is obvious.

Among the entomogenous forms, of which our knowledge is incomplete, *Sorosporella uvella* is of especial interest, not only on account of its peculiar characters, which appear to be quite unique, but because of its probable economic importance. It was first observed in Russia by Krassilstschik (*11*)[1] in 1886, and at intervals subsequently received a certain amount of attention from Russian investigators; but so far as the writer is aware it has never been recorded from other countries, despite the fact that it probably is widely distributed and destructive.

That it has not been more generally observed is without doubt due to the fact that fruiting bodies are not, as a rule, produced on the outside of the insects which are parasitized and that the insects disintegrate soon after death, leaving little that can be recognized.

It is evident from an examination of the scanty literature relating to the morphology and development of *Sorosporella uvella* that the information contained therein is very incomplete and often inaccurate, and that little or nothing is known of many phases of its development, pathogenicity, prevalence, range of hosts, etc. The object of the present paper, therefore, is to give as complete an account as possible of the stages in the life history of the fungus which have previously been neglected or inaccurately described, as well as to consider certain hitherto unrecorded phases, some of which are of considerable importance from the taxonomic, pathogenic, and even economic point of view.

It will be shown further that one type of blood corpuscles of cutworms acts as phagocytes, in that they engulf and apparently attempt to destroy the vegetative bodies of the fungus. So far as the writer is aware, the phenomenon of phagocytosis in insects attacked by fungous diseases has been observed by no one, with the possible exception of Metchnikoff (*14*), and is of especial interest, therefore, in the present connection.

The observations herewith recorded are based on studies of artificial cultures of the organism, on inoculation experiments with living hosts, and on a histological study of infected insects prior and subsequent to death.

HISTORICAL SUMMARY AND TAXONOMY

So far as the writer has been able to determine, the first description of the form under consideration is that of Krassilstschik (*11*) in Russia, who in 1886 discovered the organism within the larval body of the coleopterous sugar-beet curculio, *Cleonus punctiventris*. He referred the fungus to Tarichium, which, although it has been considered by some writers even in recent years to be a valid genus of the Entomophthorales, is in reality

[1] Reference is made by number (italic) to "Literature cited," p. 438–439."

merely a name applied to species of Entomophthora in which only resting spores are as yet known.

Although Krassilstschik's description of *Tarichium uvella* is meager and unaccompanied by figures, the measurements and characteristics of the singular resting spores which are peculiar to this parasite are clearly recorded. Those bodies are described as spherical, papillate, and from 8 to 10 microns in diameter. They are said to cohere in grapelike clusters, and to form a brick-red mass within the bodies of the dead insects. Upon germination in a nutrient solution they gave rise to septate germ tubes which bore single, terminal, cylindrical, colorless spores measuring 9 by 3 microns.

It is evident that the method of germination of the resting spores described above does not coincide with analogous processes in the Entomophthorales. Furthermore the brevity of the description served to confuse rather than aid subsequent systematists who were inclined to follow Krassilstschik in referring the organism to this family.

Two years later Sorokin (*17*, *18*), another Russian writer, found a peculiar organism in the larva of a lepidopterous insect, *Agrotis segetum*, and, apparently unaware of Krassilstschik's work, described and figured what is without doubt the same form, under the name *Sorosporella agrotidis*. Sorokin's figures are sufficiently complete and his description of the resting spores sufficiently comprehensive to render an identification possible and to indicate clearly that the form with which he was concerned was identical with that previously described by Krassilstschik.

The characteristic coherence of the resting spores in masses or aggregations was recognized by both of these authors in naming the organism, Krassilstschik expressing the condition by using the specific name *uvella*, while Sorokin selected "Sorosporella" to designate his new genus.

In 1889 Giard (*8*) called attention to the similarity of *Tarichium uvella* Krassilstschik and *Sorosporella agrotidis* Sorokin, pointing out that the characters of the two forms, such as the reddish-colored, powdery spore mass, the peculiar coherence of the resting spores, and their measurements (according to Krassilstschik, 8 to 10 microns; according to Sorokin, 4 to 7 microns) corresponded so closely that he believed them to be identical. Giard, however, in agreement with the majority of mycologists at the present time, held that the generic name Tarichium, of which the type is *T. megaspermum* Cohn., should be confined, if employed at all, to those species of Empusa and Entomophthora in which resting spores only are known. As the resting spores of the form under consideration are entirely unlike those of any known Empusa or Entomophthora, he proposed that the generic name Sorosporella be employed together with the specific name *uvella* of Krassilstschik. Giard, however, while of the opinion that the fungus should not be called Tarichium, adhered to the belief that it should be placed in the Entomophthorales.

Since the name *Sorosporella uvella* (Krass.) Gd. conforms to the present nomenclatorial rules, it will be used in this paper to designate the organism; but, as will be subsequently shown, its application to the Entomophthorales by Giard and others was undoubtedly erroneous.

Since the publication of the two papers above mentioned, descriptions have appeared of three other fungi which are perhaps identical with *Sorosporella uvella*.

Bresadola (*3*) described an entomogenous parasite under the name of *Massospora staritzii*. No illustrations were published, and the host was given as the larva of an unknown insect. The "conidial" mass was said to have been endogenous, pale flesh-colored, and the measurements of the globose or subglobose "conidia," which were provided with slightly roughened epispores, were given as 9 to 11 by 7 to 9 microns. Bresadola was unable to determine the method of formation of the bodies which he termed "conidia" because the specimen was too old.

In the following year Giard (*9*) published a note on *Massospora staritzii*, in which he expressed the opinion that this species bore considerable resemblance to *Sorosporella uvella* and suggested that the latter name be considered a synonym.

Although Bresadola's description is quite incomplete and does not mention a grouping of the resting spores, such as is characteristic of Sorosporella, it seems quite likely that *Massospora staritzii* is indeed a Sorosporella, but on account of the color of the fungus mass, which differs considerably from that of *S. uvella*, as well as the presence of a roughened epispore, the writer is inclined to consider it a different species. At this point it may be stated that the writer has in culture at the present time a species of Sorosporella, isolated from the adult of the coleopterous *Ligyrus gibbosus*, in which the fungus mass is white, and although not carefully studied as yet, its characters seem to conform more readily with those of *Massospora staritzii*. The name used by Bresadola is therefore mentioned in the present connection as a possible synonym, in order that its probable relation to the genus Sorosporella may not be overlooked.

As a second species, *Acremonium cleoni* was described by Wize (*24*) from the larva of the coleopterous insect *Cleonus punctiventris*. While Wize seems to have been familiar in a general way with *Sorosporella uvella*, which is mentioned in the introduction to his paper, he appears to have been quite unfamiliar with its development, especially the processes connected with resting-spore germination. According to Wize, *Acremonium cleoni* develops in the larvæ and pupæ of Cleonus, forming spherical, cohering cells 8 microns in diameter. From these are produced, on the outside of the insect's body, branched, septate hyphæ, some branchlets of which are bottle-shaped and bear single, terminal, ellipsoid, hyaline conidia measuring 6 by 3 microns. In artificial culture the fungus reproduces in a yeastlike fashion, first forming yellowish cells from which grow structures similar to those found on the outside of the insect's body

and bearing similar conidia. In the following description of *Sorosporella uvella* it will be seen that the method of development, including the internal formation, coherence, and size of the resting spores, the yeastlike reproduction in culture, the character of the conidiophores, the shape and size of the conidia, etc., so readily conform with analogous characters of *Acremonium cleoni*, which, furthermore, parasitizes the same insect, that there can be no doubt as to the identity of the two species.

A third species, *Fusarium acremoniopsis* Vincens (*22*), should be considered in this connection; and while the measurements of the bodies which Vincens terms its chlamydospores (3.5 by 4 microns) as well as the measurements of its conidia (4 by 7 by 2 by 3 microns) do not coincide with similar structures of *Sorosporella uvella*, there are nevertheless many points of resemblance between the two fungi which cannot be disregarded and which show, in the writer's opinion, that they, though perhaps not identical, are at least very closely related.

Vincens described his fungus as a parasite of the "ver gris" (cutworm?) from Brazil. The insect when dead was dark brown in color, but no external fructification was evident. Internally, however, Vincens found mycelia bearing short, undifferentiated conidiophores at the tips of which cylindrical, one-celled conidia were produced singly. His illustration of such hyphæ is very similar to certain structures found by the writer when *Sorosporella uvella* was grown upon artificial nutrients (Pl. 52, O). It must be noted, however, that no such structures were ever observed in cutworms infected by the same fungus.

Vincens placed fragments of the caterpillar in a moist chamber; and from these fragments conidiophores developed, the branches of which had a tendency to group themselves verticillately, a tendency, it will be shown, that is noticeable also in the conidiophores of *Sorosporella uvella*. The cylindrical spores borne on these conidiophores were largely unicellular, but a certain number were curved and provided with one or more septæ.

At a later date Vincens discovered, in dried-out fragments of the caterpillar, large numbers of brown, globular spores, grouped in spherical or oval masses, the illustrations of which resemble very closely the resting-spore aggregations of *Sorosporella uvella*, which, as the writer has indicated, are distinctive bodies and serve at once to differentiate Sorosporella from all other entomogenous fungi.

There is, therefore, considerable resemblance between *Fusarium acremoniopsis* and *Sorosporella uvella;* and in spite of the fact that the measurements of their respective reproductive bodies are dissimilar, as well as the fact that the conidia of *F. acremoniopsis* are said to be sometimes septate, the writer believes that Vincens' fungus, though perhaps not *S. uvella*, is at least some closely related species.

A few other papers, chiefly in the form of taxonomic notes, such as those of Thaxter (*21*), Danysz and Wize (*5*), and Lakon (*12*), have

appeared, in which attempts are made to define the relationship of Sorosporella with other entomogenous fungi, but on account of the scanty information afforded by the literature on the subject and the lack of original work such attempts have not met with much success.

It has been generally believed up to the present time, however, that the fungus under consideration should not be called Tarichium in the sense that the name is employed in *T. megaspermum*, but that nevertheless it should be placed in the Entomophthorales because of the resemblance of the resting spores to those of the entomophthoraceous genus Massospora.

In addition to such purely taxonomic papers, one or two have appeared dealing mainly with the artificial culture of the organism and its value as a means of combating insect pests.

Skrzhinskiĭ (*16*) apparently observed the yeastlike vegetative development of the fungus within the insect; but the significance of such a method of development was evidently not appreciated, for it was but briefly mentioned.

Danysz and Wize (*6*) report success in cultivating the organism on artificial media but deduce several erroneous conclusions regarding its life history, stating that the resting spores multiply by division within the interior of their walls, which afterward burst and release the newly formed spores. The latter continue to grow, subdividing into two or four if conditions are favorable; but if the nutrient in which they are growing becomes dried out, thick walls are produced which render them identical in appearance with the mother cells. If the cultures are kept for a sufficient time, mycelial filaments are produced from the encysted cells. Nothing is said, however, of the conidia which probably arise on the filaments. In this connection it should be stated that these authors were unable to inoculate insects either with the resting spores or with the mycelial filaments, yet they admit the infectiousness of the fungus in stating that in certain regions of Russia it is a more effective enemy of Cleonus than is *Metarrhizium anisopliae* (Oospora).

So far as the writer is aware the foregoing abstracts include all the more important references to *Sorosporella uvella* which have appeared up to the present time, and from the information therein it is obvious that the life history of the organism has been incompletely known, rendering it one of the most obscure forms of the entomogenous fungi.

Since all the descriptions of the fungus available at the present time are so brief as to be almost valueless for taxonomic purposes, the following is appended:

Sorosporella uvella (Krass.) Gd. (*8*) Syn.: *Tarichium uvella* Krassilstschik (*11*); *Sorosporella agrotidis* Sorokin (*17*); *Acremonium cleoni* Wize (*24*); ? *Massospora staritzii* Bresadola (*3*); ? *Fusarium acremoniopsis* Vincens (*22*).

Entomogenous. Resting spores formed endogenously, spherical or subspherical, 7 to 10 microns in diameter, occasionally papillate, with somewhat thick, irregular

walls; cohering in groups and arising from yeastlike, hyaline, elliptical budding cells. In mass, brick-red in color ("Brick Red" Ridgway) and powdery. Conidia thin-walled, hyaline, 4 to 6 by 9 to 11 microns in diameter, abjointed successively from bottle-shaped or almost subulate branchlets of simple or branched conidiiferous septate hyphæ and adhering after abjunction.

Hosts: *Cleonus punctiventris* Germ. (Coleoptera), Russia; *Agrotis segetum* Esp. (Lepidoptera), Russia; *Euxoa tessellata* Harr.; *Noctua c-nigrum* L.; *Agrotis ypsilon* Rott.; *Feltia subgothica* Haw.; *F. jaculifera* Guen.; and various other cutworms and noctuids in the eastern United States and Canada.

LIFE HISTORY

An opportunity was first afforded the writer in June, 1916, to study this fungus on infected cutworms received from College Park, Md. The insects showed no external signs of fungus attack even though dead, but when they were broken open a brick-red, powdery mass escaped from the larval shell (Pl. 51, A) which when examined microscopically was found to consist of spherical or subspherical, somewhat reddish-colored, moderately thick-walled cells.

Attempts were made at once to cultivate the fungus on artificial nutrients, and the usual plate isolation method was employed with potato agar as a medium. The results of the first tests were somewhat discouraging, but finally a pure culture was obtained, on another nutrient, from which subcultures have been made continuously since 1916. The following discussion is therefore based upon a study of diseased insects collected in the field, as well as on a study of insects that were inoculated in the laboratory from cultures on artificial nutrient media.

The thick-walled spherical cells as they occur within the host may be solitary, but more often they cohere in characteristic masses or aggregations (Pl. 51, H). Many show wartlike protuberances (Pl. 51, E), and others show coherent fragments of the walls of cells to which they were previously united (Pl. 51, E).

As will be shown in another connection, they may retain the power of germination for a considerable period because of their rather thick walls; and since they are, therefore, functionally analogous to similar thick-walled cells of the Entomophthorales and many other fungi, they may be termed resting spores or chlamydospores.

If a water mount is made of these resting spores and pressure is applied to the cover glass, it becomes evident that the individual cells are very firmly coherent, since their association is broken up with considerable difficulty. If sufficient pressure is applied, however, the homogeneous character of the resting-spore masses becomes apparent. The masses are resolved into their constituent cells, which prove to be undifferentiated and uniform throughout.

It should be stated in this connection that specimens collected in the field are found almost invariably in the condition just described, because the resting spores, which mark the termination of the development of

the fungus, may remain dormant for considerable periods, and when germination takes place the larval body has usually disintegrated to such an extent that it is no longer recognizable. All accounts of the fungus up to the present time have, therefore, been concerned mainly with the resting spores, although as a matter of fact these bodies represent but one phase in the rather complicated development of the organism, while an equally important phase, the production of aerial conidia, has usually been overlooked or at least not associated with the chlamydosporic condition.

Since the resting spores are thick-walled and occur within the unbroken body wall of their host, which as a rule appears to die beneath the surface of the ground, and since they are freed only by the disintegration of the host, it is obvious that they are not adapted to propagate the disease with rapidity by carrying the fungus from insect to insect, but are rather designed to enable it to survive drought and other unfavorable conditions.

To determine the origin, nature, and function of the resting spores, a number of experiments have been performed during the past two years.

In order to induce germination, freshly collected dead larvæ were placed on the surface of moist sand in covered crystallizing dishes and kept at room temperature in the laboratory. Three days later an external fungous growth on the unbroken integument of the host was distinctly visible, and after an interval of one week the latter was found upon examination to be composed of large numbers of conidiiferous hyphæ of the type illustrated in Plate 51, L. The successive stages in the germination of the resting spores and the development of the conidiophores are shown in Plate 51, F, L, N, O. Under the stimulus of moisture and suitable temperature the protoplasm of the resting spores swells, producing budlike protuberances, the walls of which are in part, at least, made up of the walls of the resting spores. The outgrowth soon assumes the shape of a germ tube, branches freely, and becomes septate. The fully developed conidiophores are supplied with bottle-shaped branchlets which show a tendency to group themselves verticillately around the main hypha. It will be further observed that conidia are borne at the tips of the bottle-shaped branchlets or sterigmata which are quite unlike the resting spores from which they arose. They measure 4 to 6 by 9 to 11 microns, are elliptical in form, thin-walled, vacuolate at each pole and are abjointed successively in the manner illustrated on Plate 51, P, cohering after abjunction.

The development and structure of the conidiophores as well as the formation of the conidia are typical of the verticillate Hyphomycetes, of which group Sorosporella should be considered a member until its perfect or acigerous condition is discovered.

The occurrence of the chlamydospores in coherent groups or aggregations recalls a similar condition that is found in the spore balls of

certain of the smuts, such as Urocystis and Tubercinia, a resemblance noted by Sorokin (*17*) in 1888. Furthermore, early stages in the germination of the resting spores in hanging drops of water (Pl. 51, C) are similar to analogous processes of promycelium and sporidium formation in some of the Ustilaginales; but although this resemblance is rather striking, it is purely superficial and of no phylogenetic significance.

The resting-spore aggregations are more naturally comparable to that class of propagative bodies known as bulbils, which, as Lyman (*13*), Hotson (*10*), and others have shown, occur in the life histories of certain Ascomycetes and Basidiomycetes. Their method of development, however, is quite different.

So far as can be determined from his imperfect description, something similar to the conidiophore production apparently was observed by Krassilstschik (*11*); and this phase of development is in itself sufficient to show that the organism can not possibly be included in the Entomophthorales, since the coenocytic hyphæ of the latter, together with the peculiar method by which their conidia are formed and discharged, are radically different and in no way comparable to corresponding characteristics of the form under consideration.

The germinations thus obtained from unbroken larvæ treated as described above were found to be confined to those spores only which lay near the surface, immediately beneath the integument. When, however, the infected larvæ were torn open and the red spore masses freely exposed to the air and light, a somewhat different result was obtained in several instances. Under these conditions the germination involved practically all the spores composing the mass, and a luxuriant growth developed quite unlike that obtained in the first instance. The differences observed were due to the fact that germ tubes from adjacent chlamydospores cohered in such a way that Isaria-like fascicles of conidiophores were produced (Pl. 51, G). Conidia were formed in the same manner and are apparently identical in every way with those described above. This type of growth does not invariably appear when infected larvæ are treated as described, but it is not unusual. The larvæ died but a few days before the tests were performed, so the resting spores were comparatively young and fresh.

The Isaria-like grouping of the conidiiferous hyphæ is somewhat similar to that which occurs in certain species of the genus Cordyceps, a condition that may be permanently conidiiferous, or represent a stage preliminary to the perfect or acigerous phase of the development. Such a condition, which was found to be parasitic upon leafhoppers (Perkinsiella) and other insects in Hawaii, and which is similar in many respects to *Isaria saussurei* that attacks wasps, has been described and figured by the writer (*19*) under the name "sterile Cordyceps." Furthermore, it has been suggested by Cooke (*4*) that *Isaria saussurei* is the imperfect

stage of *Cordyceps sphecocephala*, and the occurrence of an analogous structure in Sorosporella suggests that it also represents the imperfect stage of some species of Cordyceps or an allied type.

A second series of infections was made with army worms, *Cirphis unipuncta*, and as in the instance described above, freshly dead insects were employed. The artifically infected larvæ were, however, nearly all in the pupal stage at the time of death, the pupa case forming a thick, resistant, chitinous envelope about their dead bodies. Such pupæ were placed in moist chambers at periods varying from one week to two months after death; and although they were completely filled with resting spores which were quite normal in appearance, as was shown by subsequent examination, they produced no external conidiophores, nor did any of the resting spores germinate. When the pupa cases were torn open, however, permitting a free circulation of air about the fungus, germination was readily induced.

The results of the foregoing experiments show, therefore, that the resting spores or chlamydospores do not necessarily require a long period of rest before germination, a period that may be necessarily more or less protracted in certain other fungi, but that if suitable conditions obtain, germination may take place at once. The tests also show that when insects die in the pupal stage, germination does not take place at once unless the thick chitinous wall is broken.

In order to determine the longevity of the resting spores as well as to ascertain whether or not fresh insects could be infected with them, other tests were performed as outlined below.

Infected cutworms which died in June, 1916, were kept in the laboratory in ordinary pasteboard pill boxes until August 2, 1917, when a number of resting-spore masses were removed from them and placed in hanging drops in Van Tiegham cells. On August 6, they began to germinate; and subsequently conidiophores and an abundance of conidia were formed. This result demonstrates that the resting spores may be viable after they have been subjected to desiccatory conditions for 14 months.

In October, 1917, a number of infected army worm pupæ were buried out of doors about 2 inches beneath the surface of the soil. On March 8, 1918, they were exhumed and examined. So far as could be determined microscopically, no change had taken place; they appeared just the same as when buried the autumn before. One pupa was broken open and spore masses placed in Van Tiegham cells, while the pupa itself was placed in a moist chamber in the laboratory. On March 11 the resting spores in the Van Tiegham cells began to germinate, and conidiophores could be seen with the hand lens on the pupa in the moist chamber. The winter of 1917–18 was exceptionally severe, yet the chlamydospores were apparently unharmed. Their germination shows clearly that they are able to withstand cold as well as desiccation.

To determine whether or not fresh larvæ could be infected with newly formed ungerminated resting spores, 10 cutworms were allowed to crawl over and were rolled about in a finely powdered layer of chlamydospores in a Petri dish for 15 minutes on June 7, 1917, after which they were removed to sterile dishes in which there was a small amount of sterile moist sand. By June 25 six of the larvæ had pupated, afterward emerging. One died, but apparently not from the Sorosporella disease. On June 20 the fungus was discovered in one dead pupa, and on July 2 in another. One insect escaped during the feeding interval following inoculation. Since the larvæ were placed after inoculation in a sterile covered dish, the cover of which was removed only when fresh food was inserted, the possibility of infection from other sources seems rather remote. Although a low percentage (2 per cent) of the larvæ died from the disease, it should be noted that the deaths occurred on the twenty-third and twenty-fifth days after inoculation, whereas the normal incubation period is from 10 to 12 days, as will be shown later. As will be explained below, there occurs within the blood of infected cutworms a phagocytic reaction which may have inhibited the development of the disease, thus tending to protract the incubation period; but it seems more reasonable to believe in the present instance that infection was secured, not through the resting spores directly, but by conidia which they produced after germination; and it is obvious that the period of time necessary for the germination of the resting spores and for the production of conidia which may or may not have been in position to infect larvæ immediately after formation would readily conform with the results of the test.

Several important facts become apparent as a result of such tests, some of which appear to have a definite economic bearing. It has been shown that the chlamydospores are formed for the purpose of tiding the fungus over unfavorable conditions, since they are thick-walled and are able successfully to withstand desiccation as well as out-of-door winter temperatures. Furthermore, upon germination in hanging drops of water or in a moist chamber, they do not produce simple undifferentiated germ tubes as would be expected under these conditions, but complexed branching conidiophores, which in turn give rise to thin-walled conidia.

Although the primary function of the resting spores is that described above, it has been shown that such bodies do not necessarily require a long period of rest but may germinate soon after their formation if freely exposed to moist air. In this respect they are similar to the spores of the Ustilaginales and to the bulbils.

It is reasonable to conclude, therefore, that several generations of the fungus may occur during one season under field conditions in spite of the fact that resting spores exclusively are formed at the close of the vegetative development of the organism. This is all the more probable

because most species of cutworms habitually burrow in the soil, where they come in contact with the conidia, and because the generations of different species and even of the same species overlap and are susceptible to the disease as has been shown by inoculation experiments.

The results of the test with the army worm pupæ indicate, however, that when the resting spores are formed and retained in a thick, intact, resistant, chitinous wall such as the insect forms at this period, germination will not take place; and in fact development of conidiophores is much less luxuriant upon the untorn body wall of a larva, which, however, becomes very thin and parchment-like after a time, than on one which has been torn open. This difference is well shown on Plate 51, K, and Plate 55, A.

It is, therefore, apparent that although the germ tubes have the power of breaking out through very thin chitin, the process of germination is undoubtedly facilitated greatly when larvæ become disintegrated in the soil.

The conidia which are borne at the tips of the germ tubes are, as stated above, of a different nature from the resting spores. Their thinner walls and formation aerially in enormous numbers, as well as their general ephemeral appearance, indicate that, like analogous conidia of other Hyphomycetes, their function, quite unlike that of the resting spores, is to spread the organism rapidly and as completely as possible while favorable conditions obtain.

In a water drop, or in a drop of nutrient agar in Van Tiegham cells, germination of the conidia takes place as shown on Plate 51, J, by the production of threadlike, sinuous germ tubes. Experiments have shown (p. 433) that when conidia are placed in contact with healthy insects, either externally or internally, infection may be readily induced; and it is probable that germ tubes similar to those mentioned above penetrate the thinner portions of the body wall from without, or from within through the intestine, producing at their tips bodies which give rise to yeastlike vegetative cells. The exact method of infection, however, has not been determined, in spite of the fact that a large number of insects have been killed, fixed, and sectioned in attempts to observe germ tubes actually penetrating the insect tissues.

In the foregoing discussion the morphology of the single-walled resting spores as well as their functions and germination has been considered. It has been shown that they produce conidiophores, which in turn produce the thin-walled conidia. The production of conidia evidently marks the termination of the reproductive stage, all phases of which occur after the death of the host. The remaining stages, except the formation of the chlamydospores, are vegetative in nature and are found within the living insects after infection.

Cutworms parasitized by *Sorosporella uvella*, like insects attacked by other fungous parasites, exhibit no symptoms of disease during the

first few days of the incubation period. Three or four days prior to death, however, they become sluggish and eat sparingly; and one or two days before death a change in outward appearance may be observed. The chitinous body wall of healthy larvæ of *Feltia annexa*, one of the hosts employed, is dorsally opaque and flecked with irregular dull brown patches. Ventrally it is somewhat translucent, in fact so much so that some of its internal organs are visible. When afflicted with this disease the larvæ typically turn a creamy white color a day or two before death. If the flaccid body is pricked with a needle, a greenish or whitish liquid appears in which, if it is examined microscopically, yeastlike cells can be readily detected. These cells may be formed in enormous numbers and when abundant in the insect cause the blood to appear white, while if there are few yeast cells present the normal green color of the blood prevails.

Occasionally red-colored patches appear a few hours before death, either posteriorly, anteriorly, or, more commonly, near the middle of the body. They are more noticeable on the ventral surface because of the lack of pigment in this region. Dissections show that mature resting spores are present in such areas; and their early occurrence and maturity in such spots possibly indicate the seat of infection, although, as noted below, the detached yeastlike cells which later develop into resting spores are distributed throughout the body cavity by the blood of the insect in such a way that a local early maturity of the chlamydospores would seem impossible.

In one or two instances the whole middle portion of the body of an infected insect became reddish and noticeably shrunken, the anterior and posterior ends retaining the normal greenish, turgid appearance. The prolegs of such diseased portions showed no reaction when pinched with a pair of forceps, but the true legs at the anterior part of the body reacted when similarly treated.

With the exception of such symptoms, which, as has been stated, occur only in the later stages of the disease, there are no others prior to death, so far as the writer is aware, which indicate the presence of Sorosporella.

Death follows a day or two after the natural pigment of the insect disappears; and the creamy white color soon changes to pink, the latter usually appearing simultaneously over the whole body and becoming more and more intense until the final development of the organism, indicated by the brick-red color, is reached. The disease can thus be readily recognized at any time after death without the aid of a microscope.

In contrast to most known species of entomogenous fungi (except certain species of Entomophthora which form resting spores only), Sorosporella completes its entire development within the body of its host, producing no growth externally.

Shortly after death the body appears shrunken and wrinkled. It is somewhat flattened, and there is nearly always a longitudinal, ventral,

furrow-like depression present in its abdomen. The body, while not limp or flaccid, is soft and pliable; and if a portion of its skin is indented, it remains sunken with little or no reaction. The body is never hard and sclerotium-like as in insects infected with *Botrytis rileyi*, for example. If pricked with a needle no liquid emerges. If torn open entirely, the internal fungus mass is seen to be quite coherent and of a shiny creamy or pink color and gelatinous consistency. A convoluted, almost vermiform structure of the spore masses can easily be recognized with a hand lens.

Later in the development of the organism the host's body becomes more shrunken and the reddish color more intense, but there is no other change in outward appearance. The body wall is now quite brittle, the slightest shock serving to rupture it. The moist, gelatinous character of the spore masses has disappeared; and they become typically brick-red in color, dry, dustlike, and less coherent than before. Absolutely nothing remains of the internal organs of the host, and in fact the body appears as, *17* Sorokin (*18*) first suggested, very much like a minute sac filled with dust.

The fungus is found in this stage of development when collected in the field in summer, and according to Danysz and Wize (*6*) it may be seen in the same condition after having overwintered in the soil, a statement, it will be recalled, that is in accord with the writer's findings.

In the foregoing paragraphs the known symptoms of diseased insects, as well as the gross characters of the fungus during the incubation period and the post-mortem aspects of the disease, have been considered. To observe the microscopic characters of the vegetative stages of the fungus, however, many infected specimens have been examined when alive and when killed, fixed, and sectioned. During the first few days of the incubation period it is quite impossible to recognize the organism in any of the insect tissues. Upon examination of the blood of infected insects on the sixth or seventh day after inoculation, however, yeastlike cells will be observed floating free in the blood lymph mingled with the blood corpuscles. These yeastlike cells, as will be subsequently shown, form the early vegetative states in the development of the organism; and on account of their similarity to yeasts, the name blastocysts will hereafter be employed to designate them.

The cells when young are quite regularly elliptical in form, hyalin, measuring 8 microns by 5 microns, and in blood smears occur singly or coherent in pairs, rarely in threes; (Pl. 52, I; 53, B). A preparation made from the blood of a diseased insect on the sixth day after inoculation will show but few of these bodies, but on the seventh day they become more numerous, and on the eighth and ninth days, under normal conditions, they are strikingly conspicuous, vastly exceeding in numbers the blood corpuscles, which may themselves be abundant. They multiply within the blood plasm by a yeastlike germination, all stages of

which can be seen in one preparation. Sometimes at one end, sometimes at both ends of the blastocyst a budlike outgrowth or papilla appears which grows rapidly until it assumes the form and approximate size of its parent. For reasons given below it soon breaks away from the parent cell and proceeds to form new cells by a similar process. At times a mucronate outgrowth appears at the tip of the blastocyst instead of a papilla, so that a short neck is formed, at the distal end of which a daughter cell arises (Pl. 52, I).

Multiplication continues in this manner until enormous numbers of blastocysts occur within the blood plasm; and since they are free-floating, the circulation of the blood distributes them uniformly from place to place, so that they are found throughout the body cavity, within the heart (Pl. 55, B), and in all regions where it is possible for the blood to penetrate. The nature of the blastocysts and their somewhat loose attachment to one another is such that the circulation of the blood must evidently be considered the essential factor in spreading them to all parts of the body as well as in aiding vegetative reproduction.

The blood lymph becomes so loaded with blastocysts that not only is its progress eventually impeded but its function of supplying oxygen to the organs of the insect is apparently inhibited. As a result of what is perhaps lack of oxygen, the host acts as though stupefied; and, though alive, it responds to stimuli feebly, remaining in a comalike condition for several hours before death.

When the insect dies or when the blood circulates slowly before death, the blastocysts, though formed in the usual manner, no longer break away from one another; instead of isolated cells, colonies of coherent individuals are formed (Pl. 51, D), which ultimately develop into the resting-spore masses. At the same time that the colonies are formed, however, the blastocysts individually change from an elliptical to nearly spherical form. Since each blastocyst is able potentially to form new colonies, a large number of the latter are produced; but many do not grow to large size, evidently because the nutrient in the blood is exhausted. Other small colonies coalesce so that large spore masses are formed, and still others that are in close proximity to fat bodies or other sources of nourishment are able to grow as long as this nutrient lasts.

The writer has never seen cells of the parasite actually intruding into the insect organs, and in fact the budding process of growth is such that intrusion would not be expected. On the contrary there appears to be a substance secreted by the fungus which causes the organs to break down; and, as would be expected, those which are in contact with the fungus colonies disintegrate first.

The chitinous body wall of larvæ in which resting spores are fully mature is always very thin (Pl. 51, K), and even the tracheæ are broken down completely, fragments of the taenidia only remaining; whereas in insects in which blastocysts are beginning to develop the tracheæ are

intact, and the body wall, except after molting, is very thick. When resting spores are transferred from freshly opened larvæ to artificial media pure cultures are invariably obtained, indicating that no extraneous organisms are present in the body cavity; and the fact that the chitin has a tendency to disintegrate in the presence of Sorosporella indicates that this fungus secretes a solvent of some kind.

Before the chitin breaks down, however, all the softer tissues disappear, among the first of which are the fat bodies, the membranous walls of which disintegrate readily (Pl. 52, A).

A colony of fungus cells which is closely opposed to a fat cell will bud off more freely nearest the source of nourishment; hence, after the wall of the fat body has broken down at the surface, the colony will tend to enter and take the form of the organ, a portion of the wall of which persists for some time. The result of such development is that lamella-like or vermiform convoluted colonies are formed.

Although the fat bodies disappear first as a result of the growth of the fungus, the muscles, nerve fibers, malpighian tubes, and all the hypodermal tissues gradually succumb, until eventually only fragments of the tracheæ can be observed. Even the alimentary tract disappears, and its position is indicated merely by fragments of food.

As the colonies of the fungus continue to grow, the individual cells gradually become larger and secrete single, rather thick walls about themselves, so that ultimately aggregations of spherical, rather thick-walled, cohering cells are formed, which were described above as resting spores or chlamydospores. The formation of resting spores marks the end of the vegetative development of the fungus. These spores are in fact only modified blastocysts, though they are themselves reproductive.

The discovery of the yeastlike cells or blastocysts, the method of development and mode of life of which have been considered above, and the fact that they represent the entire vegetative stage of the organism under consideration, is of considerable scientific importance. Although found by the writer for the first time in connection with Sorosporella, similar bodies were observed by De Bary (*2*) associated with other entomogenous fungi, a fact quite unknown to the author until these investigations were completed.

De Bary found what he called "cylindrical conidia"—processes analogous to what have herein been called blastocysts—free-floating within the blood of various insects infected with *Botrytis bassiana*, *Isaria farinosa*, and other similar fungi. He observed that these bodies, though sometimes drawn out to two or three times their original length, usually remained elliptical in form and gave rise to secondary and tertiary conidia on short sterigmata. Such a method of multiplication was followed until the blood at the expense of which the organism developed was full of "cylindrical conidia," when, because of the large numbers of

these bodies present, it became milky white in color. He also noted that the circulation of the blood was an important factor in the distribution of the blastocysts, and that it gradually became absorbed, so that when an insect was pricked with a needle in later stages of the disease no liquid emerged. When the cylindrical conidia had completed their development, however, instead of rounding up to form chlamydospores, as do the similar bodies of Sorosporella, the bodies were observed by De Bary to lengthen out, become septate and branched, and otherwise assume the form of branching hyphæ.

It should be stated, however, that the "cylindrical conidia" were considered by this author to be homologous with those formed in the air, for he says (translation):

> The cylindrical conidia are typical organs of our fungus whose development is aided or reduced according to the nature of the surrounding medium, not dependent upon it, for they arise constantly as the first product of the germ tubes, in the air as well as in a fluid medium.

While this may be true with *Botrytis bassiana*, which the writer has not yet studied in detail, the blastocysts of Sorosporella obviously can not be homologized with its aerial conidia, because the power which the blastocysts possess of reproducing their kind by yeastlike budding is not shared by the conidia, which furthermore are quite different in form.

De Bary also calls attention to the fact that Audouin (*1*) as early as 1837 probably saw the cutting off of cylindrical conidia in the blood of silkworms infected by *Botrytis bassiana*, and that, according to Robin (*15*), Guérin Ménéville undoubtedly found them, though erroneously claiming that they "arose from the granules contained in the blood corpuscles." On account of their supposed origin Guérin Ménéville called them "Hæmatozoidia," a name which can not be employed at the present time in the light of more recent investigations.

While these earlier authors undoubtedly saw the free-floating fungus cells, Vittadini (*23*), according to De Bary (*2*), first realized their significance because he followed them through the various stages in their development more closely than did any of his predecessors.

Recent literature, however, is entirely lacking in reference to these peculiar free-floating vegetative cells; and only when the older papers were examined by the writer, after these investigations were completed, did it become evident that they were not entirely unknown among entomogenous fungi.

Through the lack of special study of their vegetative phases, as well as ignorance of the older literature, it has been generally supposed in recent years that entomogenous Hyphomycetes as a class vegetate within insects by branching hyphæ. It is possible that such a method of growth obtains in certain entomogenous species, but in *Sorosporella uvella*, *Isaria farinosa*, and *Botrytis bassiana* bodies are formed which seem especially

adapted for reproduction in a liquid menstruum such as insect blood, the circulation of which carries them to all parts of the body cavity and continually supplies them with fresh nutriment.

Thaxter (*21*) in 1888 showed that in certain cases at least the vegetative development of the entomogenous Entomophthorales was carried on not by branching hyphæ, but by "hyphal bodies" which consisted of short, thick fragments of irregular size and form that reproduced by budding, a phenomenon analogous to that described above, though the blastocysts of Sorosporella are regularly elliptical and symmetrical in form.

Because of the similarity of the blastocysts to yeasts and in certain stages even to sporozoans, considerable confusion might result and erroneous conclusions be reached if subsequent stages in the development of the fungus were not known; and in fact so anomalous in character were the blastocysts that considerable care was taken to prove their identity with Sorosporella.

In the course of the cultural experiments described below, it was found that when Uschinsky's solution was inoculated with Sorosporella, a type of yeastlike cell was produced that is quite comparable with that found in the blood of insects. This similarity will at once be evident by comparing Plate 53, B, with Plate 53, A. The former is a photomicrograph of the blastocysts produced on Uschinsky's solution and was made from a water mount, while the latter is from the blood of an infected insect and was made from a slide stained in Erlich's haematoxylin and eosin.

In order to obtain further evidence, however, single blastocysts were removed from the blood of an infected insect by means of Barber's pipette holder and were transferred to nutrient agar.

A small area on the abdomen of an infected insect was washed in alcohol, after which a sterile capillary tube was inserted into the body, and a small amount of blood together with a number of blastocysts was collected. The tube was then removed and its contents ejected upon an inverted sterilized glass slide placed upon a suitable holder on the stage of the microscope. Examination of the drop of blood was then made with the microscope. A place was chosen where a small number of blastocysts occurred, and another sterile capillary tube fastened to Barber's holder was adjusted in such a way that only a few blastocysts were drawn into it. These blastocysts were ejected upon the slide at another place and the process repeated until only one blastocyst was drawn into the tube. This single fungus cell was then ejected upon a hanging drop of culture medium, and a pure growth of Sorosporella resulted.

The operation, though requiring a certain amount of care, was not especially difficult in the present instance, because no extraneous organisms were found in the blood to interfere, and because the blastocysts, which are large, are readily visible and easy to manipulate.

PHAGOCYTOSIS

During the course of these investigations a phenomenon was observed, which, though well known in animal pathology, has received little or no attention from insect pathologists. The discovery of this phenomenon, phagocytosis, in cutworms infected with *Sorosporella uvella* may be of considerable importance from the economic as well as the scientific point of view; and while it is realized that the information given herewith is far from complete, because the subject of phagocytosis and especially its relation to immunity is not clearly understood, it is hoped that the evidence submitted will show clearly that the vegetative bodies of Sorosporella are ingested by the leucocytes of the infected hosts, a condition that has generally been overlooked in previous investigations of insect diseases.

That such a condition has been overlooked is without doubt due to the fact that the vegetative development of entomogenous fungi—that is, those phases which occur within living insects—have never been studied in much detail, although the external reproductive phases are in many cases well known.

When it was discovered that the fungus under consideration vegetates within the blood of infected insects during the early stages of the development, blood smears were made; and after the usual preparatory methods they were stained in Erlich's haematoxylin and eosin. In addition to variable numbers of blastocysts, such slides always show large, spherical, or spindle-shaped, free-floating cells which are of insect origin. Stained preparations showing these cells were submitted to Dr. W. A. Riley, of the University of Minnesota, who kindly informed the writer that he considered them typical blood corpuscles, or leucocytes. He recognized four types, all of which are representative of the Lepidoptera: (1) Proleucocytes, small cells, with large nuclei and little cytoplasm; (2) phagocytes, larger fusiform cells with central nuclei; (3) spherule cells, rounded, vacuolate, and with irregular nuclei; and (4) oenocytoids, large nonphagocytic cells with dense protoplasm.

Of those four types only one, the fusiform cells, is of interest in the present connection, since they alone seem to be phagocytic. These will be considered below under the general terms leucocytes, or blood corpuscles, or the more specific term phagocytes.

When blood smears of cutworms infected by Sorosporella are prepared with the stains noted above, the vegetative budding cells of the fungus are made clearly visible. Not only do they occur often in great abundance, floating free within the blood plasm, but many may also be observed firmly and distinctly imbedded within the cytoplasm of the leucocytes (Pl. 52, B–E), a condition that had escaped attention in water mounts, in which there is of course no differentiation of contents of the leucocytes.

An examination of large numbers of stained slides which were made from several infected insects renders the statement possible that such a condition is of regular occurrence in such insects during the later stages of the incubation period, though it is not possible to state at the present time that every infected cutworm shows this phenomenon.

When treated with the stains noted above, the fungus cells, or blastocysts, are pink. The leucocyte cytoplasm is light blue, whereas the nuclei of the leucocytes are dark blue; hence the fungus cells are clearly differentiated in the blue-stained matrix of the blood cell cytoplasm.

The number of blastocysts contained within a single phagocyte varies from 1 to 15, and so far as can be determined they are in all respects similar to those which occur floating in the blood plasm and may even be seen in the process of budding off new cells (Pl. 52, B, *a*). When gorged with large numbers of fungus cells the phagocytes become abnormal in form and size, though the tail-like protoplasmic appendage can usually be recognized. If but one or two fungus cells are inclosed, however, the form of the leucocyte is unchanged, and its cytoplasm seems likewise to be little affected; for it stains as deeply as in uninfected cells. On the other hand, the cytoplasm of blood corpuscles in which several blastocysts are imbedded seems to be in part destroyed, for it stains feebly. It should be noted, however, that although the nuclei of the infected cells may be compressed or even distorted, they seem to remain intact, staining brilliantly in all tests. The fact that those leucocytes with large numbers of blastocysts imbedded in their substance seem to show evidence of disintegration, together with the fact that in the many preparations examined no evidence of the breaking down of the blastocysts has been observed, renders the conclusion possible that the fungus cells gradually destroy the cytoplasm of the blood cells, which in many cases so completely breaks down that nothing remains.

Such a sequence of events is not unusual in human diseases. There are many cases on record in which it is evident that the phagocytes fail in their attempt to destroy the invading organism, the latter being the more potent; but the act of ingestion of the parasite by the leucocytes is considered nevertheless as phagocytosis, though it may be ineffective so far as the destruction of the parasite is concerned.

In addition to the stained blood smears, paraffin sections were cut of infected larvæ which were killed and fixed in Carnoy's solution. The sections were stained in Erlich's haematoxylin and eosin as before, and in them the phenomenon of phagocytosis was made evident in another way (Pl. 52, J, N).

Complexes such as those referred to are composed of certain cohering cells. Those near the center of the mass are more or less fused, or at least are compressed so closely against one another that their individual identity is hard to determine, though their nuclei remain distinct. Those cells near the periphery of the complex, however, retain their

individuality to some extent; and from an examination of such free or semifree individuals it is possible to determine that they are identical with the free-floating, spindle-shaped cells of the blood, or in other words the phagocytes.

The phagocytes are often arranged in irregularly concentric layers about a mass of free blastocysts, or in other instances infected leucocytes seem to act as a focus around which numbers of blood cells, for the most part uninfected, gather (Pl. 56, A, B). Other sections show what may be called compound complexes, in which there are two or three foci, or centers of attraction, the whole being surrounded by a common envelope composed of several layers of leucocytes. These aggregations, or complexes, occur throughout the body cavity, though more commonly perhaps near the heart or tracheæ; and although they are of various sizes and shapes, they tend to be roughly spherical.

The fusion or coalescence of the amoeboid-like leucocytes recalls at once the similar action of the amoebæ of Myxomycetes in forming plasmodia; and furthermore, as in the Myxomycetes, this coalescence is not accompanied by nuclear fusions, for the nuclei remain distinct.

Since the phagocytic action of the blood corpuscles of susceptible hosts upon the vegetative bodies of Sorosporella had been observed, it was deemed advisable to determine whether or not similar action would occur when the organism was introduced into the blood of non-susceptible hosts.

In inoculation experiments it was found that when the usual methods of infection were employed, silkworms (*Bombyx mori*) and white grubs (*Lachnosterna* spp.) did not succumb to the disease. Sorosporella conidia were therefore injected within specimens of these insects by means of a hypodermic needle. The needle was made from a piece of 4-mm. glass tubing, one end of which was drawn out into a very fine point. An ordinary atomizer bulb was attached to the other end. Conidia in suspension in sterile water were drawn into such sterile tubes, and the body of the host was punctured. Then pressure was applied to the bulb, forcing the conidia into the body cavity. It was found necessary to fasten the insects to some substratum in order to prevent them from wriggling and rupturing the organs around the needle. The needle was then quickly removed; and if the operation was carefully performed, very little blood escaped.

The technic here employed was so crude that it was impossible to to measure very carefully the amount of fluid injected into the larvæ. The diameter of the needles averaged less than 1 mm., and a column of fluid 0.5 cm. long was considered a small dose.

When small injections were made it was nearly always possible to observe conidia in the blood of silkworms and white grubs a few hours after injection, in prepared blood smears; but one or two days after inoculation it was impossible to detect fungus cells of any sort in such

smears. However, although the conidia had disappeared, no evidence of their incorporation by leucocytes was obtained in spite of the fact that these bodies were quite abundant. A few insects so treated were not subsequently punctured to get blood smears but were left unmolested, and after a period of two weeks they were still alive.

It is not possible to say whether the conidia were destroyed by the blood antibodies, or whether they were taken up by fixed cells, which of course would not be seen in blood smears; but in any event their disappearance is suggestive of some such action, and the fact that certain control insects similarly treated survived further indicates that the fungus cells were in some way rendered impotent.

On the other hand, when a large dose of the fluid was injected into silkworms and white grubs, first conidia and then blastocysts could be detected in the blood in due time after injection. The blastocysts appeared within one or two days after injection; and so far as could be determined they reproduced in a way identical with that in susceptible hosts. Similarly the act of phagocytosis was recognized; but, as in susceptible hosts, the phagocytes seemed unable to cope with the fungus, the vegetative bodies of which were formed in enormous numbers. Furthermore the blastocysts rounded up and ultimately formed typical resting-spore aggregations.

In order to determine whether or not there is at first an active phagocytosis in infected susceptible hosts, conidia of Sorosporella were injected into specimens of the semitropical army worm (*Prodenia eridania* Cram.) in small and large doses in a manner similar to that described above.

Blood smears were made from such insects at periods varying from two hours to three days after injection. While it was possible to detect both the fungus cells and the phagocytes in all stained blood smears whether or not such smears were made from insects into which a larger or smaller amount of the fluid was injected, phagocytosis was not observed within two days after inoculation although careful search for infected leucocytes was made. On the other hand, smears made two or three days after injection showed blastocysts incorporated in the phagocytes, although no signs of disintegration of the former could be detected. Furthermore, it is certain that the fungus cells were reproducing rapidly in the blood, for specimens in the 2-celled stage of division, such as are shown in Plate 52, I, were in great abundance.

A detailed discussion of Metchnikoff's discovery of phagocytosis in Daphnia, a crustacean, and his subsequent formulation of the theory of phagocytosis as an explanation of immunity in man and other animals is not within the scope of the present paper, but it seems advisable to consider such phases of this and other theories as may have a bearing on the problem under consideration.

The theory set forth by Metchnikoff (*14*) stated that when an animal is attacked by a hostile organism its blood corpuscles and other proliferating cells of mesodermic origin ingest and destroy the parasite. For a number of years this theory together with the Humoral theory have been considered the two most plausible explanations of immunity; but unfortunately each attempted to explain this phenomenon from a point of view that seemed to be directly opposed to the other, the Humoral theory holding that hostile organisms were destroyed by a body fluid in the nature of a serous exudate.

These differences appear to have been somewhat adjusted in recent years by the work of Erlich, Denys and Leclef, Wright, and others, which has shown the body fluids and the phagocytes to be interdependent in certain human diseases. Wright, notably, proved that the act of phagocytosis is dependent, at least in some diseases, upon the presence of certain substances in the blood, and that these substances (opsonins) act upon the invading organisms, not necessarily killing them, but rendering them susceptible to ingestion by the leucocytes.

It is believed by a certain group of men at the present time that the act of phagocytosis is directly associated with the process of immunization, although the destruction of hostile organisms is brought about not by the phagocytes alone but with the aid of certain body fluids, like opsonins, the nature of which is not fully understood at the present time. Other factors probably are important, such as the potency of the parasite; but these can not be considered here.

While it has been shown in certain human diseases that the leucocytes actually ingest parasitic organisms in a manner comparable to that described above, it is nevertheless well known that such ingestion may end in the destruction of the phagocytes as well as in the death of the organisms, the result apparently depending upon the relative resistance or potency of the leucocyte and parasite.

A study of the blood of infected cutworms has shown that the blastocysts of the parasitic fungus occur within the cytoplasm of the leucocytes. The process by which this position is obtained has not been observed in living material, but from a study of stained individuals it is clearly indicated that amoeba-like pseudopods arise from the phagocytes by means of which the blastocysts are incorporated into the substance of the leucocyte. The occurrence of the phagocytes in aggregations or cysts, however, indicates that some sort of attraction exists between the insect cells and the fungus cells. It is well known that mobile protoplasmic cells exhibit certain definite movements when subjected to mechanical, chemical, or thermal stimuli; and in view of the discovery of Wright, it seems likely that some substance is present in the blood plasm of infected cutworms which not only renders the blastocysts susceptible to ingestion but which also may exert some sort of attraction, perhaps chemotactic, upon the leucocytes, rendering the formation

of aggregations or complexes possible. Furthermore, since the blood corpuscles possess amoeboid movements and are carried from place to place by the circulation of the blood, the formation of the cystlike aggregations is easily understood when this attractive force, perhaps chemotaxis, is considered.

Whatever the force may be, the fact remains that plasmodia are formed, evidently for the purpose of destroying the fungus cells. It has been shown, however, that the latter are more potent than the leucocytes, which ultimately disintegrate; so that while the act of phagocytosis is undoubtedly present and may impede the progress of the disease in cutworms, it can not be considered a successful defensive process in the present instance.

The same statement may be made in regard to those nonsusceptible insects into whose bodies a large number of conidia were injected and which, it will be remembered, were unsuccessful in combating the fungus. When a small number of conidia were injected into the blood of such nonsusceptible hosts, however, it is evident that something was present which was sufficiently potent to render the fungus cells innocuous.

From the discovery of phagocytosis in cutworms parasitized by Sorosporella, it is reasonable to assume that it may be present in other insects attacked by other fungi. It is furthermore not unlikely that the relative potency of the phagocytes and fungi may vary according to the insect and fungus.

Those who have ever attempted to spread entomogenous fungi in the field in an attempt to control insect pests have invariably found many problems relating thereto apparently inexplicable. A further study of phagocytosis may assist in solving these questions. It may explain, for example, why certain individuals or species are immune and others susceptible and why the period of incubation may vary to such a degree in the same disease. From a broader point of view it is not impossible to believe that it will likewise explain why insects appear more susceptible under certain apparently favorable weather conditions than under unfavorable conditions.

CULTURE OF THE ORGANISM

Immediately upon the receipt of the first infected cutworms from College Park, Md., attempts were made to cultivate the organism on artificial nutrients. The plate separation method was at first employed to isolate the fungus; but later it was found, as has been previously noted, that pure cultures could be readily obtained by transferring groups of resting spores directly, with a sterile needle, to sterile plates or tubes.

When first transferred from its natural host to artificial media the organism grows very slowly, and on potato agar it develops less luxuriantly than on any other medium employed. Unfortunately potato

agar was the first nutrient to be used, and it was subsequently discarded as unsuitable. Its use for the first cultures led almost to failure, for some of the plates were discarded after a period of 10 days, when there appeared to be no growth. Fortunately other plates were retained for a longer period; and after 2 weeks a few mycelial strands were observed which could be traced to the spore aggregations, but impurities crept in and crowded out the slow-growing Sorosporella. After numerous fruitless attempts, however, pure cultures were obtained on other more suitable media, from which subcultures have since been made without difficulty.

Fawcett (7), in cultivating the entomogenous Aschersonias of the white fly in Florida, met with the same difficulty; he found that such fungi grew very slowly on artificial nutrients. In fact, he attributes failure in the past in cultivating the species of Aschersonia to the fact that plates were discarded before the organisms had time to develop.

Although a number of media have been employed, several of which are suitable, the writer now uses Molisch's culture media for luminous bacteria[1], modified to some extent, because it is readily made and seems well suited for Sorosporella. This nutrient will hereafter be called Molisch's agar or Molisch's solution.

After the fact was established that the fungus under consideration could be successfully cultivated artificially, a variety of nutrients were employed, on several of which its development was found to differ somewhat from that which occurs in infected insects. Furthermore, single conidia and single resting spores were isolated by Barber's pipette holder and placed in a nutrient drop of agar in a Van Tiegham cell, where development from day to day was easily noted; and pure cultures were subjected to different environmental conditions in order to determine the optimum temperature for growth of the organism as well as its behavior in unusual atmospheres.

To determine the optimum temperature a number of tube cultures were inoculated at approximately the same time. A few were placed in an incubator, a few in a water cooler, and others were kept in the laboratory. The temperature of the incubator varied from 35° to 40° C., that of the water cooler from 18° to 20°, whereas that of the room ranged from 22° to 35° during the month that the tests were in progress. In the incubator the fungus developed feebly though in a normal manner, the thallus covering an area no greater than a square centimeter one month after inoculation. In the water cooler, on the contrary, growth was very luxuriant; and within the same space of time the fungus had spread over the entire surface of the agar slants. The room temperature for the first week or so was quite high, varying between 32° and 34°, and growth of the organism did not keep pace with that in the cultures in the cooler; but later when the room temperature dropped the fungus began to

[1] Water, 1,000 cc.; agar, 15 gr.; sugar, 20 gr.; peptone, 10 gr.; dipotassium phosphate, 0.250 gr.; magnesium sulphate, 0.250 gr.

vegetate more freely, so that at the end of one month cultures left in the laboratory showed nearly as luxuriant a growth as those retained in the water cooler.

The results of these tests indicate a preference of Sorosporella for temperatures varying from 18° to 22° C.; and the fact that such lower temperatures are preferred is not surprising, since the fungus under natural conditions grows in insects which are rarely exposed to heat above 22°, cutworms, for example, being exposed to the air only at night when it is cool, and resting during the day under boards or other débris where the temperature is somewhat lower than in more exposed places.

The fact that the natural vegetative development of the fungus occurs within a liquid menstruum, insect blood, the aeration of which is supposed to be more or less complete through the respiratory action of the tracheæ, led to attempts to cultivate it in artificial liquid media, in certain tubes from which the air was largely removed or replaced by another gas.

In fermentation tubes of Molisch's solution to which the air had free access, however, no gas formation could be detected; but yeastlike vegetative cells were formed abundantly, which were quite similar in size and form to those formed within living insect blood. With the exception of soft Uschinsky's agar and soft Molisch's agar, no other nutrients have been found upon which the fungus vegetates typically by blastocysts; and it seems probable that such a type of development is aided by and perhaps even dependent upon a liquid, or at least a very wet substratum. In addition to the blastocysts which occurred within the nutrient solution and at some distance from its surface, a complex of mycelium filaments, composed of slender, threadlike, septate, sinuous hyphæ, as well as thick filaments with large barrel-shaped cells, was formed at the surface of the solution. The slender, sinuous threads gave rise to conidiophores and conidia; but the thick, barrel-shaped cells first formed spherical, red-colored resting spores, although these bodies later germinated, giving rise to conidiophores and conidia.

To determine whether or not the organism would grow in a partial vacuum, Molisch's solution was placed in ordinary test tubes into which pieces of glass tubing led through perforated rubber corks. The glass tube was then heated about half way from either end and drawn out until it was considerably constricted. The exposed end of the glass tube was then closed with a cotton plug. After sterilization such test tubes were inoculated, and a water pump was attached to the glass tubing with a wash bottle interposed. By allowing the pump to work a few minutes a sufficient amount of air was removed from the test tube to render a fair vacuum. When this was accomplished the constricted portion of the glass tube was rapidly sealed and divided by the application of a Bunsen flame. Several tubes were treated in this manner, in none of which was there at any time afterward any apparent growth of

the organism, but although a limited amount of air seems to be required, free circulation of air is not necessary, because, as noted above, blastocysts were found to develop in fermentation tubes at some distance below the surface of the nutrient solution.

To grow the fungus in an atmosphere of nitrogen instead of air, Buchner's method was employed. Small test tubes filled with Molisch's solution and inoculated were placed in larger test tubes, the latter having been previously filled to a depth of 1 cm. with pyrogallic acid. A few cubic centimeters of a strong solution of caustic soda were then poured quickly between the tubes, the larger of which was immediately sealed with a rubber stopper. The action of the caustic soda upon the pyrogallic acid renders the latter alkaline and in so doing extracts the oxygen from the air, leaving an atmosphere that is composed largely of nitrogen.

When subjected to the conditions outlined above, Sorosporella exhibited no signs of growth whatever; and it may be concluded, therefore, that an atmosphere of nitrogen inhibits growth.

Upon a favorable nutrient and when subjected to the usual cultural conditions in the laboratory, the development of the fungus under consideration takes place somewhat as follows: Mature resting spores when sown either in Van Tiegham cells or in Petri dishes may give rise directly to conidiophores and conidia, as in a drop of water (Pl. 51, L); or at times vegetative, budlike outgrowths are produced, such as are shown at the bottom of M in Plate 51. Such budlike processes produce enormous numbers of cells that remain attached to one another and form a colony. After a short period the members of this colony round up and then give rise to conidiophores and conidia. The conidia, however, when sown on nutrient agar, put forth slender germ tubes (Pl. 51, Q), which after a time become septate and branch profusely. In about 10 days a colony is formed, in which, in addition to such slender hyphæ, there can also be detected large, thick, many-septate fungus filaments, the cells of which are more or less barrel-shaped. In young cultures it can readily be determined that such hyphæ arise as branches of the slender threads. An examination of a colony 2 weeks after the conidia are sown will show, therefore, two types of filaments which are so thoroughly intermingled that it is impossible to ascertain the origin of either. Many of the slender hyphæ increase greatly in length, and those which are at or near the surface of the nutrient agar give rise to sterigmata of the type illustrated in Pl. 52, O. Usually the sterigmata are sessile and occur irregularly on the prostrate hyphæ, which often show a tendency to group themselves in bundles. Occasionally upright, much-branched conidiophores are produced of a type quite similar to those arising from the germinating resting spores.

The thick barrel-shaped cells, however, although at times giving rise to slender, prostrate, vegetative filaments, usually grow more nearly

spherical in form and give rise to other cells of the same nature by a proliferating process, ultimately resulting in a growth of the type illustrated in Plate 51, S. Again, under conditions not fully understood, the elements of such a proliferating colony may become largely disassociated and reproduce by a yeastlike budding process. When either of the last two methods of development is followed the cells normally round up and become thick-walled, forming bodies that have been called resting spores. These bodies are homologous to those found in diseased cutworms and resemble them in the distinctive red color which is visible to the naked eye. On account of the fact, however, that the cells which are differentiated to form resting spores may germinate prematurely in culture and to the fact that the red color is associated only with mature, thick-walled chlamydospores, it should be stated that such pigmentation is not always apparent, or it may appear in limited areas in a culture and not be present elsewhere.

As a result of these diverse methods of development, cultures are produced which to the naked eye present quite different appearances, differences that seem to be due entirely to the tendency of the chlamydospores to germinate in situ at any time during the process of their formation. A comparison of Plate 54, B, with Plate 54, A, will illustrate such different types of growth; and while these photographs are from cultures on beerwort agar and Molisch agar, respectively, dissimilarity is often just as apparent in the same culture. The thallus shown in the latter is convoluted, creamy white, and woolly. It is composed for the most part of immature resting spores which have germinated in situ and have remained attached to one another. In such a proliferating process of growth, if new cells, whether produced laterally or terminally, remain attached to their parents and are incompletely abstricted, a toruloid growth results, the older cells of which germinate in situ before they are matured, forming sterigmata and conidia. When several closely opposed colonies develop in this manner, a thallus is formed which resembles that illustrated in Plate 54, A.

If, on the other hand, the new cells which are formed become largely disassociated from their parents, a growth results that is somewhat different in gross appearance. Under such conditions growth is typically yeastlike, and the elliptical detached cells enlarge considerably by the absorption of nourishment and bud off new cells, so that eventually heaped-up, grapelike bunches are formed. Continued swelling renders the cells nearly spherical in form. Thick walls are deposited. When viewed with the naked eye the characteristic red color invariably associated with fully formed resting spores is at once apparent. Plate 54, B, will illustrate the type of thallus that results from such a method of development, although the photograph was taken a few days too late to show it to the best advantage because germination of the resting spores had already begun in the older portions of the culture.

It should be noted, however, that in some cultures a growth appears such as is shown in Plate 54, B, only to be superseded a few weeks later by one such as is shown in Plate 54, A, which indicates that there is a pronounced tendency on the part of the fungus to change from its natural method of yeastlike development toward a filamentous habit when it is grown on ordinary 2 per cent agar nutrients. When, however, a very soft agar or a liquid nutrient is employed as a substratum, the normal yeastlike habit has a tendency to persist.

Under certain other conditions, however, the tendency to produce free elliptical cells followed by mature resting spores has been very pronounced, as, for instance, in slanted tubes in which the brainlike, convoluted type of development has taken place in a number of instances about the point of inoculation, to be followed some weeks later by the formation of grapelike bunches of mature red resting spores near the bottom of the tube where the agar had shrunk away from the glass.

As noted above, the fungus under consideration was cultivated on a variety of media; and while its development in certain respects was similar on all, differences were observed which are, perhaps, of sufficient worth to be noted.

POTATO AGAR

The development of Sorosporella upon potato agar is always feeble; colonies in flasks do not reach a diameter of 4 mm. by the end of two weeks. After a growth of four or five weeks, a dense, prostrate, dirty white surface mycelial weft is formed, which is composed of very fine, hairlike hyphæ bearing conidia sparsely. The colony at this time may be 1 cm. in diameter. In two or three weeks more it reaches a diameter of 2 to 3 cm. and a dark brownish-colored area appears around its periphery. Growth then seems to cease; the brownish area becomes almost black and extends farther into the medium, forming a dense black ring 2 mm. wide around the colony. A microscopic examination of this area in which no surface mycelium can be seen shows that the discoloration is due to a pigmentation of the subsurface mycelium. True resting spores do not form, though the early stages of the Torula-like budding process can be seen. Transfers of the fungus from such cultures to Molisch's agar have shown that the organism is viable after five months.

MOLISCH'S AGAR

The composition of Molisch's agar, which has been successfully used for growing many species of fungi, is given on page 423. Sorosporella grows more luxuriantly on it than on any other medium employed, except perhaps beerwort agar. A photograph showing its peculiar gross habit after five weeks growth is given in Plate 54, A. When flasks are inoculated by the streak method the fungus may appear in one or both of the two ways that were considered on pages 425–426. Colonies formed by the

yeastlike budding of the fungus cells are at first round, glistening, and white. Later they are red, of a gelatinous consistency, and form grapelike, heaped-up pustules. As noted above, such pustules are composed of mature resting spores, and when they germinate the red color of these bodies is in part obscured by the mass of conidiophores produced. Colonies formed by the toruloid method of growth are convolulted, dense, homogenous, and cream-colored; and after coalescing with other colonies they form a growth such as is shown in Plate 54, A. The crateriform portions of the thallus result largely from a tangential division of the fungus elements within mechanically fixed borders. As noted above, the resting spores in such colonies do not always reach maturity but germinate, giving rise to short, subulate sterigmata, on the tips of which groups of conidia are formed. The sterigmata give the thallus a woolly appearance.

BEERWORT AGAR

The first series of cultures of Sorosporella which were made on beerwort agar developed in a way that has since been observed only occasionally. That is to say, the yeastlike budding process of development, resulting in grapelike aggregations of mature red resting spores, took place in all cultures to the exclusion of all other methods of development. The resting spores did not germinate until they were almost or entirely mature, so that the inoculated flasks presented a striking appearance, the richly developed thallus being exclusively red in color. A portion of a streak culture is reproduced in Plate 54, B; and although the photograph was taken a few days too late, when the resting spores had started to germinate, it is possible to recognize in certain portions of it the gelatinous grapelike clusters of these bodies.

CORN-MEAL AND OAT AGAR

The development of Sorosporella is essentially the same on corn-meal and on oat agar, on both of which it presents some phases that are quite unlike those on other nutrients. At first the writer was inclined to class these nutrients with potato agar as undesirable; but by keeping certain cultures for a long period, a growth resulted that is especially worthy of note. Subsequent inoculations have shown that this peculiar phase of development occurs regularly. As on potato agar, growth is at first extremely slow, practically no surface mycelium being formed around the point of inoculation. After a period of six weeks, however, small wartlike protuberances appeared at the bottom of the tubes, being more abundant in regions where the nutrient had shrunken away from the glass, and at some distance from the point of inoculation with no surface mycelium intervening. The wartlike protuberances, in growing, gradually coalesced, forming a prostrate, crustlike thallus, the red color of which at once indicated the presence of chlamydospores. These resting

spores germinated, not by giving rise directly to sterigmata, but first to hyphæ, those from adjacent spores cohering. As growth proceeded, erect fascicles of hyphæ were thus produced which reached a height of from 1 to 5 mm. Such a synnema is illustrated on Plate 51, G. This fasciation always appears on corn-meal and oat agar if time enough is allowed. It has not been observed on any other nutrient except Uschinsky's. It is worthy of mention because it is identical with the growth that sometimes appears when an infected cutworm is opened up and the inclosed resting spores allowed to germinate on moist sand (see Pl. 55, A). Although such cultures were kept for several months, no further development was observed except that the conidiophores changed from white to a rusty brown in color.

USCHINSKY'S SOLUTION WITH AGAR [1]

For two weeks after inoculation there was no apparent growth of Sorosporella on Uschinsky's solution with agar as a medium. As is often the case with freshly made cultures, however, condensation water was present in the tubes. When the tubes were handled this surface liquid now and again washed against the bit of fungus thallus that was used to inoculate the tubes, and in three weeks' time this surface liquid became milky white in color and upon microscopic examination was found to be full of yeastlike fungus cells. A water mount was made of these cells, a photograph of which may be seen in Plate 53, B. Later the surface liquid changed to a light brown tint and became viscid in consistency. Without changing in other respects it gradually became darker brown and more viscid. The growth was so typically and exclusively yeastlike, liquid, and unlike anything before seen that a bit was transferred to Molisch's agar tubes to determine whether it was an impurity. In due time, however, typical grapelike clusters of red resting spores appeared, indicating that these yeastlike bodies were in fact the organism in question. In the meantime no change had taken place in the original tubes; but at the end of two months the yeastlike elliptical cells began to assume a spherical shape, and when viewed with the naked eye a reddish tinge of the thallus was observed.

The viscid, yeastlike growth gradually disappeared and was superseded by a heaped-up, pustule-like development, more intensely red in color. This growth was composed of spherical, thick-walled resting spores. For a considerable period there was no further change in appearance of the cultures except that as additional resting spores developed the heaping-up process became more conspicuous. Then germination of the resting spores began, and erect fascicles of conidiophores were formed in a manner comparable to that noted above. It is worthy of

[1] Agar agar 15 gr.; asparagin 3 to 4 gr.; ammonium lactate 10 gr.; sodium chlorid 5 gr.; magnesium sulphate 0.2 gr.; calcium chlorid 0.1 gr.; dipotassium phosphate 1 gr. Dissolve in 1,000 cc. water and add 40 cc. glycerin.

remark that throughout the entire vegetative development of the fungus on this medium nothing resembling hyphæ was observed, the entire process having been exclusively yeastlike.

Several months after these tests were conducted a fresh supply of this agar was made up, and on it the fungus under consideration developed in a manner in all respects similar to that described above.

The artificial cultivation of Sorosporella has shown, therefore, that it can be successfully cultivated on a variety of media, and that while its normal method of vegetative development within infected insects is by means of yeastlike budding cells, this habit may be modified by cultivation to such an extent that a semifilamentous growth may be acquired. The cultures also show that when the resting spores reach maturity before germination—a condition that is never accomplished when the toruloid or filamentous habit is followed but which does occur as a result of growth of the disassociated blastocysts on certain nutrients—fascicles of conidiophores arise which recall similar fascicular growths of other insect fungi, such as those of the poorly defined genus Isaria. It should be noted furthermore that no perfect or acigerous stage has been observed in any of the artificial cultures.

INOCULATION EXPERIMENTS

Although the morphological characters, particularly of the resting spores, had been studied to some extent prior to the writer's preliminary work with the fungus in question (*20*), attempts to inoculate insects artificially, either in the laboratory or in the field, were rarely recorded, in spite of the fact that in Russia at least the disease was known to occur under natural conditions out of doors in such abundance and in such a manner as to suggest its infectiousness.

It was deemed advisable, therefore, to perform a series of inoculation experiments to determine, if possible, something of the range of susceptible hosts and the pathogenicity of the organism, as well as to determine a method of infection that could be used in a practical way to inoculate large numbers of insects.

The successful artificial culture of the fungus greatly facilitated the work of conducting these tests, for a constant supply of viable conidia was at all times available. Since it was determined that insects could be readily infected by the fungus from artificial cultures, the latter were invariably used.

It has been shown in the foregoing pages that the vegetative development of the fungus takes place exclusively within the blood of its insect hosts; and it is obvious that to reach the body cavity, the body covering, the tracheæ, or the intestine must be penetrated by the conidial germ tubes. As is well known, insects are covered externally by a layer of chitin, which in certain regions, as for example between the body seg-

ments, is relatively thin. Furthermore, it is generally stated that this layer of chitin extends into the mouth and anus, lining the intestine in both these regions. There is, however, a relatively small portion of the intestine, the mid gut, which is supposed to be of different origin from the fore and hind gut, and which is said not to be lined with this substance. The tracheæ, a ramifying system of spirally reinforced tubes opening externally in the spiracles, are likewise lined with chitin, though this substance is exceedingly thin, especially in the ultimate portion of the tubes.

It is apparent, therefore, that of the regions where the germ tubes might enter the body cavity, all save the mid gut are protected by an inert, resistant substance through which the germ tubes must penetrate. For this reason especial care has been taken to examine the mid gut in prepared sections of inoculated individuals.

It was deemed a matter of considerable interest, both from the scientific and economic points of view, to know exactly how and where infection takes place; and several tests have been carried out with the view of determining this point. There are certain portions of the body wall in which the chitin is comparatively thin, such, for example, as the intersegmental membranes, the flexible leg joints, etc. Furthermore, the glandular openings and hair follicles are apparently less perfectly adapted to resist the penetration of the conidial germ tubes than are most parts of the body. Likewise it would seem quite possible for the small conidia to gain entrance into the body through the spiracular openings of the tracheæ, and once inside to germinate and cause infection. The intestine is, as stated above, lined for the most part with chitin, though this substance is relatively thin in most places and entirely absent in the mid gut.

With these regions of possible infection in mind, artificial inoculations were arranged in such a way that conidia came in contact with all portions of the external body wall and the intestine, and bits of fungus mass were also carefully placed on the tip of a needle directly in contact with the spiracles. In order to introduce the conidia into the alimentary tract, cutworms were fed with clover leaves which had been smeared with the fungus; and to place the conidia in contact with all external parts of the body wall, a bit of conidial agar paste on the tip of a needle was rubbed about over the body, although to facilitate subsequent microscopic examinations of sections of the larvæ it was often applied particularly to the dorsal and ventral medial lines of the body.

To examine the tracheæ after inoculation, it was found advantageous to dissect these organs from the body and mount them directly in alcohol. Although a large number from many insects were examined microscopically, the writer was quite unable ever to find conidia in them. The examination revealed, however, the presence of chitinous spiny bristles at the opening of the spiracles and clustered at various points along the lumina of the tracheæ, and when these were found it became evident that they would effectively prevent the entrance of conidia.

To examine the various parts of the intestine and the body wall, however, it was necessary to cut serial sections of the body. The insects were killed and fixed in Carnoy's solution in periods varying from one to six days after inoculation, after which they were prepared by the usual methods and stained in Erlich's haematoxylin and eosin, such stains having been found of value in differentiating blastocysts within the blood cells.

Although hundreds of sections from a large number of insects were examined, it has been impossible to observe the conidial germ tubes penetrating either the body wall or the intestine. When insects were fed with leaves smeared with conidia, it was possible actually to observe the fungus entering the mouth; yet sections cut of such individuals did not show conidia even in the lumen of the alimentary tract, in spite of the fact that such insects were killed and fixed before the conidia-bearing leaf fragments were voided. In certain slides, however, blastocysts were observed within the folds of a tissue connecting the longitudinal and transverse muscles of the intestine; but it could not be determined whether this position was attained by entrance of the blastocysts from the body cavity or the intestine (Pl. 52, K). Similarly no conidia were observed externally penetrating the body wall of the inoculated insects, though special care was taken to examine the regions noted above and in spite of the fact that in many cases the conidial spore paste was applied in definite regions to facilitate examination.

That the fungus does gain entrance into the body cavity is obvious, however; and as the following tests will show, it seems probable that both the body wall and the intestine may be penetrated by the conidial germ tubes. Inability to observe them is perhaps due to improper time of killing or faulty technic.

To test the parasitism of the fungus and to determine a method of infection that would be adapted to the inoculation of insects artificially on a large scale many tests were conducted of a more general nature than those noted above. A variety of insects, representative of nearly all the larger orders, were inoculated by one or sometimes by all of the methods described below. The hosts used were as follows:

Diptera—larvæ of *Musca domestica* L.
Coleoptera—larvæ of Lachnosterna, larvæ of Elateridae.
Orthoptera—nymphs and adults of grasshoppers.
Isoptera—workers of *Reticulitermes* sp.
Lepidoptera—larvæ of *Feltia jaculifera* Guen., *Feltia subgothica* Haw., *Peridroma saucia* Hübn., *Agrotis ypsilon* Rott., *Noctua c-nigrum* L., *Chloridea obsoleta* Fab., *Hyphantria textor* Harr., *Leucania unipuncta* Haw., *Phlegethontius sexta* Joh., *Bombyx mori* L., and several other members of the family Noctuidae.

Although the fungus has been collected in the field in this country upon two species of Euxoa only, all the Noctuidae used in the laboratory tests, including the corn earworm and army worm, were shown

to be susceptible to the disease. The other species of Lepidoptera—*Hyphantria textor*, *Phlegethontius sexta*, and *Bombyx mori*—did not die of the disease when they were inoculated by the usual methods, yet when conidia were injected into the blood of *Phlegethontius sexta* and *Bombyx mori*, by means of a capillary pipette, death followed; and when such insects were examined, it was found that blastocysts had developed in the blood in a normal manner.

In general, the inoculations were made by one of the three methods outlined below, and after infection the insects were placed in sterile battery jars, partly filled with sterile sand, or if a record of specific insects was desired, they were isolated, one each in small sterile boxes. Control larvæ were kept with all experiments.

DIRECT CONTACT METHOD

Larvæ were placed in flasks or bottles of 500-cc. capacity in which there was a well-developed fruiting surface of the fungus. They were allowed to remain therein for varying periods of time in the several tests and were therefore constantly crawling about over the thallus. It was realized that insects in the field would probably be in contact with the source of infection only for a moment; therefore, in order to simulate in artificial cultures conditions as they occur in the field, the length of time which the insects were allowed to remain in contact with the fungus in the laboratory tests was limited in certain cases to one or two minutes, although in other instances the insects were left in the inoculating flasks for several hours.

In other experiments bits of the fungus thallus were removed from cultures with a sterile needle and rubbed upon the body of the larvæ, or in other instances small portions of the thallus were placed in small boxes on the top of a layer of moist sand and fresh larvæ were then inserted into the boxes. The fungus was allowed to remain in such receptacles for several days so that the cutworms were in daily contact with it.

It is obvious, therefore, that although the methods of inoculation varied in detail, the principle underlying all was the same and consisted in allowing larvæ to come in direct contact with the fungus.

In the discussion of the morphology of the conidia the fact was mentioned that they cohere after abjunction, indicating that a substance is secreted which renders them viscous. This substance is not secreted as profusely as in certain other verticilliacious Hyphomycetes, however; the little heads of conidia were never observed involved in mucus. It is obvious that a substance of this sort would serve also to fasten the conidia to the bodies of insects with which they came in contact; and if certain of the conidia found lodgment in regions suitable for their further development, it is reasonable to believe that infection would result. It is apparent, however, that when the cutworms were allowed

to crawl over the fungus thallus for several hours, the conidia may have been ingested in some numbers; and in such instances the ingested conidia may have produced the disease; but on the other hand, in those instances in which the fungus was applied to the external parts of the body only, it seems necessary to believe that infection was produced by penetration of the body wall by the conidial germ tubes.

The larvæ of all species of cutworms were quite well developed when inoculated, pupating in several instances in a short time. It is worthy of note in this connection that a number of the army worms employed were in the pupal stage at the time of death, and that a few imagos emerged, lived a day or two, and then died of the disease. The occurrence, therefore, of typical resting spores in imagos of the army worm is significant and indicates that the organism, though unable to kill the larvæ and pupæ, passed through the various metamorphic changes of the host and finally caused death after it emerged. The presence of the fungus in the flying adults is furthermore significant, since it suggests the possibility of dissemination of the organism. It should be noted, however, that the wings of the four adults from which the fungus was recovered were imperfectly formed, and other organs were malformed or missing. An antenna of one, for example, was entirely lacking, and a portion of the leg of another was absent, indicating that during the metamorphosis certain of the imaginal tissues were destroyed.

During the summers of 1917 and 1918, 20 tests were performed by the direct contact method, in which many insect species were used; and while it is not desirable to discuss all of them, a few may be considered.

On April 9, 1917, 26 larvæ of *Feltia jaculifera* were inoculated by allowing them to remain in the culture flasks for 12 hours. Thirteen died of Sorosporella on April 20, 8 on April 23, and 2 on April 24. On May 1 when the experiment was closed 3 were alive. There were 12 larvæ in the control dishes, 1 of which died from Sorosporella on April 20. The others were alive on May 1. This larva is the only one that died of the disease in control dishes during the course of all of the experiments.

On August 3, 100 army worms were inoculated by being allowed to crawl over the fungus for two minutes or less. On August 6, 8 larvæ were dead and on August 10, 9 more, from some other cause than Sorosporella. On August 13, however, the fungus was recovered from 9 insects. Metarhizium had killed 2, and 14 had died from unknown causes. On August 15, Sorosporella was found in 20, and 2 were dead from other causes. On August 16, 13 more were dead from Sorosporella; on August 20, 7 emerged, and there were 5 dead from which Sorosporella was not recovered. Finally on the date the test was closed, August 23, 4 adults were found infected with the fungus, as well as 7 pupæ, all of which were filled with resting spores. Of the 25 control larvæ, 5 died from unknown causes and 20 emerged.

In another test, May 9, 15 larvæ of *Feltia* sp. were inserted in as many salve boxes in which were placed moist sand and a bit of fungus

thallus. On May 14, 1 larva died from some unknown cause, but on May 21, 12 larvæ died of the fungus. On the two succeeding days 2 other larvæ died of the same cause. In the control dish in which 10 larvæ were inclosed, 9 were alive on May 23 and 1 had died from an unknown cause.

As an example of those inoculations in which the larval bodies were rubbed with a bit of the fungus, an experiment begun on June 29 with 23 larvæ of *Noctua c-nigrum* may be cited. Thirteen of these were inoculated and 10 were used as controls. On July 6, 3 larvæ died in which the fungus could not be detected. On July 13, 14, and 15, however, 5, 3, and 2 insects, respectively, had died from the disease; and at the close of the test on July 22, 7 larvæ were alive in the control dish, 3 having died before July 15 from unrecognized causes.

In addition to a large number of similar tests that were conducted with noctuid larvæ of various species the direct contact method was also used in inoculating house-fly larvæ, white grubs, wireworms, nymphs and adults of grasshoppers, and white ants, as well as the larvæ of *Hyphantria textor*, *Bombyx mori*, and *Phlegethontius sexta*. The insects in all cases were placed in culture tubes or flasks of the fungus and allowed to remain therein for from 3 to 4 hours, after which they were removed and placed in boxes with suitable food.

The house-fly larvæ, for example, when treated in this manner were found to be covered with conidia when they were removed from the cultures, yet after they had burrowed in the dung of the rearing boxes for a few hours no conidia could be detected with the low power of the microscope. While several specimens, particularly of *Hyphantria textor* and house-fly larvæ, died from unknown causes, the fungus was not recovered from a single inoculated insect of any of the species enumerated above.

The experiments with the susceptible hosts show, however, that under laboratory conditions a high percentage of mortality may be realized and, furthermore, that the death rate is not appreciably higher when larvæ are kept in contact with the fungus for a long time than when they are subjected to infection for a minute or two.

The length of time necessary for the fungus to kill nearly mature cutworms is rarely less than 10 days; and in certain instances it was longer than 10 days, a possible explanation for which is given on page 417. This last summer, however, smaller larvæ in the second and third instars were inoculated for sectioning purposes, and it was found that such insects often succumbed to the disease in 6 or 7 days.

SPRAY METHOD

Ten cc. of sterile water were poured into a culture flask, which was then shaken until a quantity of conidia were in suspension. The liquid was then sprayed upon healthy larvæ by means of a small hand atomizer,

and after inoculation the insects were placed in sterile boxes in the manner described above.

Microscopic examination showed that the liquid held quantities of conidia in suspension. Many conidia therefore must have lodged on the insects, though the liquid did not spread in an even pellicle over the body but gathered in little droplets. Control larvæ were sprayed with sterile water only and were subsequently removed to sterile boxes.

On May 5, 10 larvæ of *Feltia* sp. were inoculated. On May 14, 1 died from an unknown cause; on May 21, 1 died of Sorosporella; and by June 16, 8 were alive. In the control experiment of 10 larvæ, 2 died on May 29 of unknown causes, and the remaining 8 were alive on June 16 at the close of the test.

Seventy-five army worms were inoculated on August 6, and 25 were held as controls. Three of the infected insects died on August 10, but the parasite was not found in them. On August 13, 3 of the inoculated insects, as well as 2 of the control larvæ, died from Metarhizium; 18 of the inoculated specimens had died from unknown causes; and 6 of the control larvæ were dead. On August 17, the parasite was recovered from 5 larvæ. On August 20, 22 of the infected ones emerged; 8 were dead from Sorosporella, and 8 from other causes. Finally on August 23 the remaining 7 of the inoculated army worms were dead from unrecognized causes, and 17 of the control larvæ emerged as adults.

Several other tests of a similar nature were conducted, but it is not advisable to consider them here. The foregoing examples are sufficient to show what may be expected of this type of inoculation. The average mortality of all the tests, however, was somewhat below 30 per cent; and the experiments as a whole serve to corroborate an opinion already formed by the writer in connection with other entomogenous fungi, to the effect that the spray method is of less value in artificially inoculating insects than the direct contact method.

The reason for this is not apparent, because hundreds of conidia must have lodged upon the infected insects. Furthermore, the conidia germinate freely in water and when sprayed upon insects they were apparently in a suitable position to insure infection.

FEEDING METHOD

In order to introduce the fungus into the alimentary tract of cutworms, a conidial agar paste was smeared over clover leaves, which the caterpillars were allowed to eat. But first the leaves were cut into small portions not larger than 5 square millimeters in order to reduce to a minimum the possibility of contact between the leaves and the external parts of the insect's body. Such leaf fragments were then placed in sterilized Petri dishes, into which were also inserted fresh larvæ, one to each dish. Many of the larvæ were hungry, for they had purposely been unfed for several hours; and the process of eating was followed in many

instances until the small bit of leaf was entirely consumed. It is therefore possible to say that the fungus did not come in contact with any external portion of the body except the mouth parts, and the first and second pair of legs, which grasped the leaf, and held it edgewise to facilitate feeding. All conditions under which the experiments were conducted were carried out in such a manner as to preclude the possibility of infection from other sources; and since the fungus at no time came in contact with the body except in the manner stated above, it is obvious that infection was brought about through the ingestion of food.

During the summer of 1918 a number of inoculations were made in this manner. The hosts used were largely cutworms of various species, chiefly *Prodenia eridania* Cram.; and a death rate was obtained in all instances that was as high as in those inoculations which were made by the direct method. A detailed account of one experiment may be given. Twenty-five army worms were fed on August 7 with clover leaves which had previously been smeared with conidia from an agar culture. As a control, 15 larvæ of the same lot were fed with fresh, unsmeared leaves only. On August 10, 3 of the infected specimens and on August 12, 2 of the control larvæ died from unknown causes. On August 16, however, 10 of the infected army worms were dead, in all of which the fungus was recognized; and 3 were dead from other causes. On August 15, 2 and on August 20, 3 larvæ died in the control dishes, but apparently not from the Sorosporella disease. On August 17, 4 and on August 19, 2 of the infected insects died from the disease, and 1 died from which the fungus was not recovered. The experiment was closed on September 3, when 2 larvæ of the infected lot and 8 of the control lot were alive. In spite of the fact, however, that the conidial germ tubes must obviously pass through the intestinal wall, it has been impossible actually to observe them in prepared stained sections.

SUMMARY

(1) The presence of *Sorosporella uvella*, an entomogenous fungus, is recorded for the first time in America.

(2) The previous association of Sorosporella with the Entomophthorales is shown to be erroneous, and the proper position of the organism, among the verticilliacious Hyphomycetes, is designated.

(3) The reproductive bodies are thick-walled resting spores or chlamydospores and thin-walled conidia, the latter being herein definitely associated with the life history of the organism for the first time.

(4) It is shown that yeastlike vegetative cells, existing within the blood of infected insects, are ontogenetically related to other phases in the development of the organism.

(5) There is an ingestion of these vegetative cells by certain of the blood corpuscles (phagocytosis), the process being apparently followed by

the destruction of the phagocytes. This phenomenon has, up to the present time, been overlooked by those investigators who have studied the fungous diseases of insects.

(6) The organism is readily cultivated on artificial nutrients and exhibits two quite different types of growth when grown on favorable media.

(7) In certain cases, both when the fungus was grown on media and when the resting spores were placed in a moist chamber, fruiting structures of the Isaria type developed.

(8) No perfect or acigerous condition has been observed.

(9) The disease caused by the organism is readily transmitted to healthy insects, and in laboratory experiments a mortality of from 60 to 90 per cent may be readily obtained.

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PLATE 51

Sorosporella uvella:

A.—Infected cutworm torn open, exposing the resting-spore aggregations. × 1.2.

B.—A single resting-spore aggregation. × 115.

C.—A portion of a resting-spore aggregation germinating in water, showing promycelial-like germination, and conidia. × 250.

D.—A colony of young resting spores from an infected insect, showing the manner in which they reproduce. × 570.

E.—Isolated mature resting spores, some of which show the ruptured walls of previously cohering spores. × 570.

F.—Mature resting spores germinating in water. × 570.

G.—Isaria-like fascicle of cohering conidiiferous hyphæ which developed when the resting spores of an infected larva were allowed to germinate in a moist chamber. × 300.

H.—Portion of a mature resting-spore aggregation. × 250.

I.—Conidia, or secondary spores. × 570.

J.—Conidia, or secondary spores, germinating. × 570.

K.—Portion of a section through the body of an infected cutworm which had been placed in a moist chamber to induce germination of the resting spores, showing the usual type of conidiophores. × 200.

L.—Mature resting spore germinating in water, showing conidiophore with verticillately arranged sterigmata. × 570.

M.—Mature resting spores germinating on nutrient agar, showing sessile sterigmata and conidia at one place and young resting spores arising by budding at another place. × 570.

N.—Early stages in the germination of mature resting spores in water. × 570.

O.—Advanced stages in the germination of resting spores in water. × 570.

P.—Sterigmata, showing method of conidial abjunction. × 570.

Q.—Enlarged view of conidial germination. × 1,050.

R.—Enlarged view of conidia. × 1,050.

S.—Torula-like reproduction in culture. × 570.

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PLATE 52

Sorosporella uvella:

A.—Portion of a fat body from an infected insect which is being destroyed by an adhering group of young resting spores. × 190.

B, C.—Phagocytes from an infected cutworm with blastocysts of the fungus imbedded in them. × 570.

D, E.—Phagocytes distorted in form and partially disintegrated as a result of the action of the enclosed blastocysts. × 570.

F.—The terminal portion of a complexly branched hypha from nutrient agar, showing sterigmata and conidia. × 570.

G.—Normal blood corpuscles of *Feltia jaculifera*. × 570.

H.—Resting spore germinating on nutrient agar, showing hyphæ, some of which are producing resting spores. × 225.

I.—Blastocysts, showing method of reproduction. × 1,050.

J.—An aggregation of cohering leucocytes in the substance of some of which blastocysts are to be seen. × 550.

K.—Portion of the intestine of an infected *Feltia jaculifera*, showing longitudinal and transverse muscles connected by a frail membrane within the folds of which blastocysts may be seen. × 650.

L.—Colony of young resting spores from a culture, which are giving rise to hyphæ. × 225.

M.—Colony from a Petri dish culture, showing a mass of young resting spores, some of which have germinated in situ by giving rise directly to sterigmata and conidia, and others that are producing hyphæ. × 225.

N.—Small phagocytic complex. × 550.

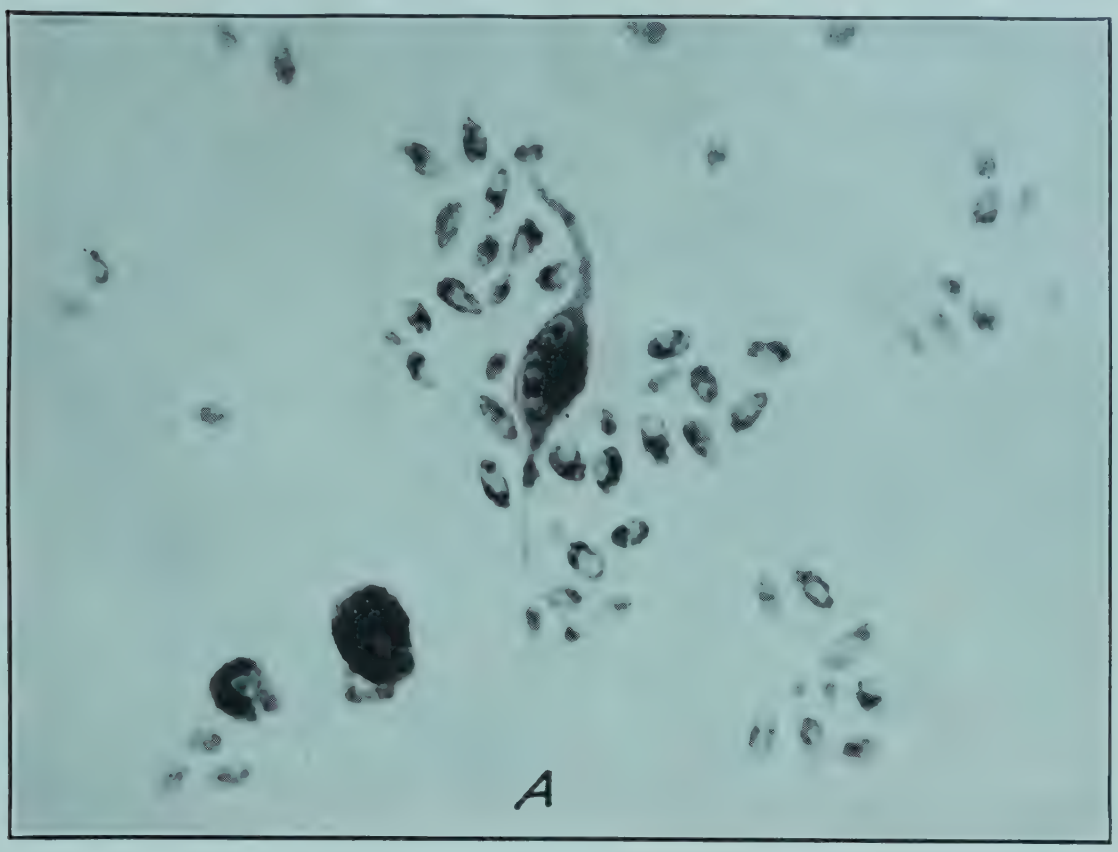
O.—Portion of one hypha rising from a resting spore that had germinated in a Petri dish culture, showing sterigmata and conidia. × 550.

PLATE 53

Sorosporella uvella:

A.—Photomicrograph of a stained blood smear from an infected cutworm, showing a number of free-floating blastocysts and one leucocyte within which are to be seen two blastocysts. × 600.

B.—Photomicrograph of a water mount of blas tocysts which developed on Uschinsky's medium after inoculation. × 600.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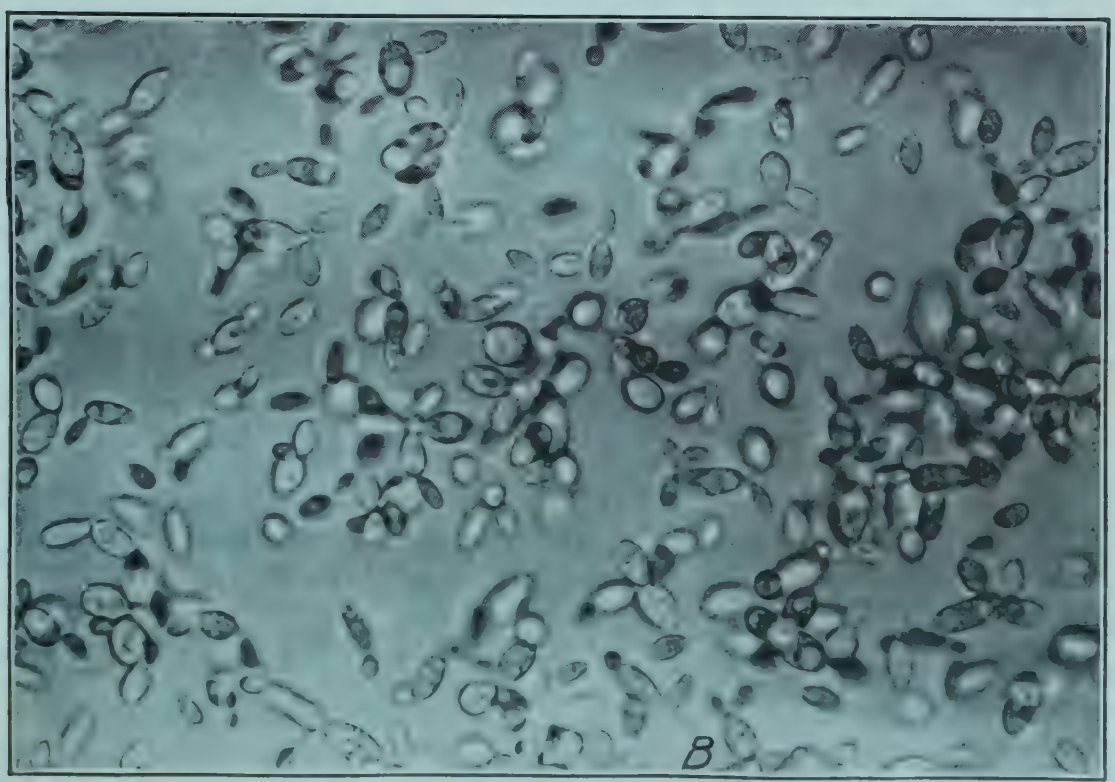
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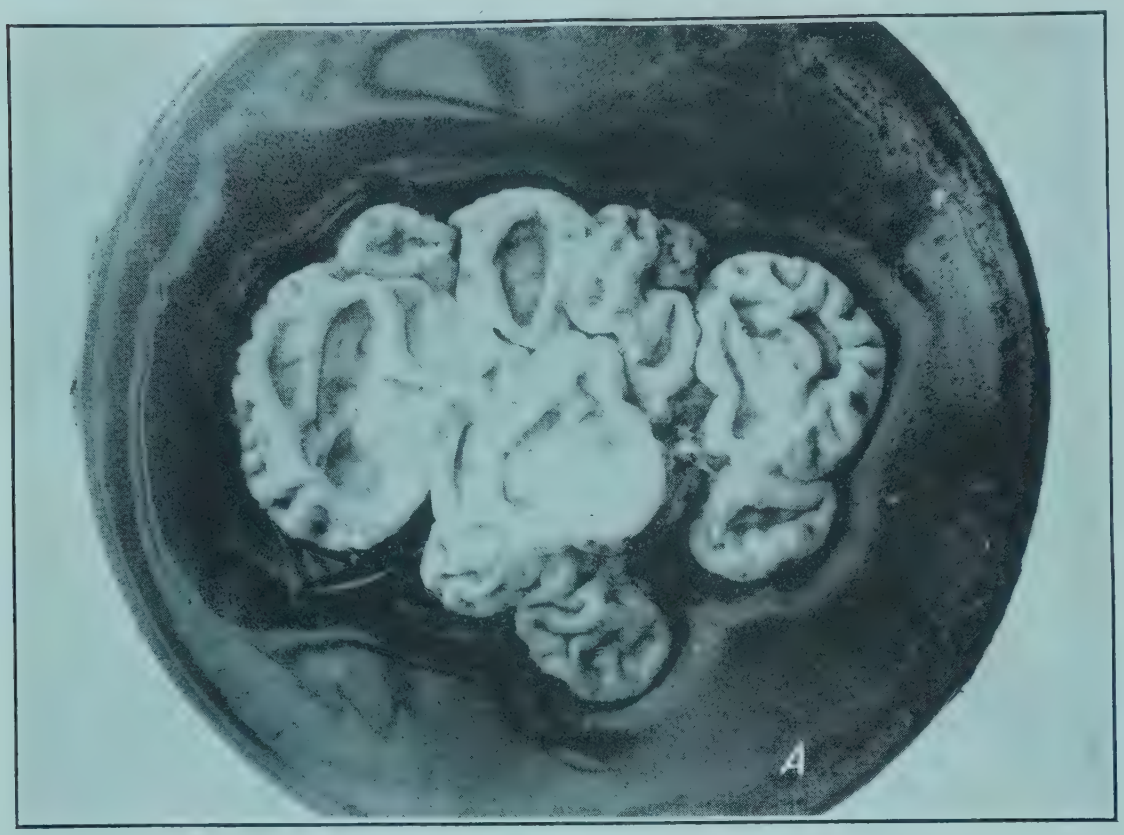
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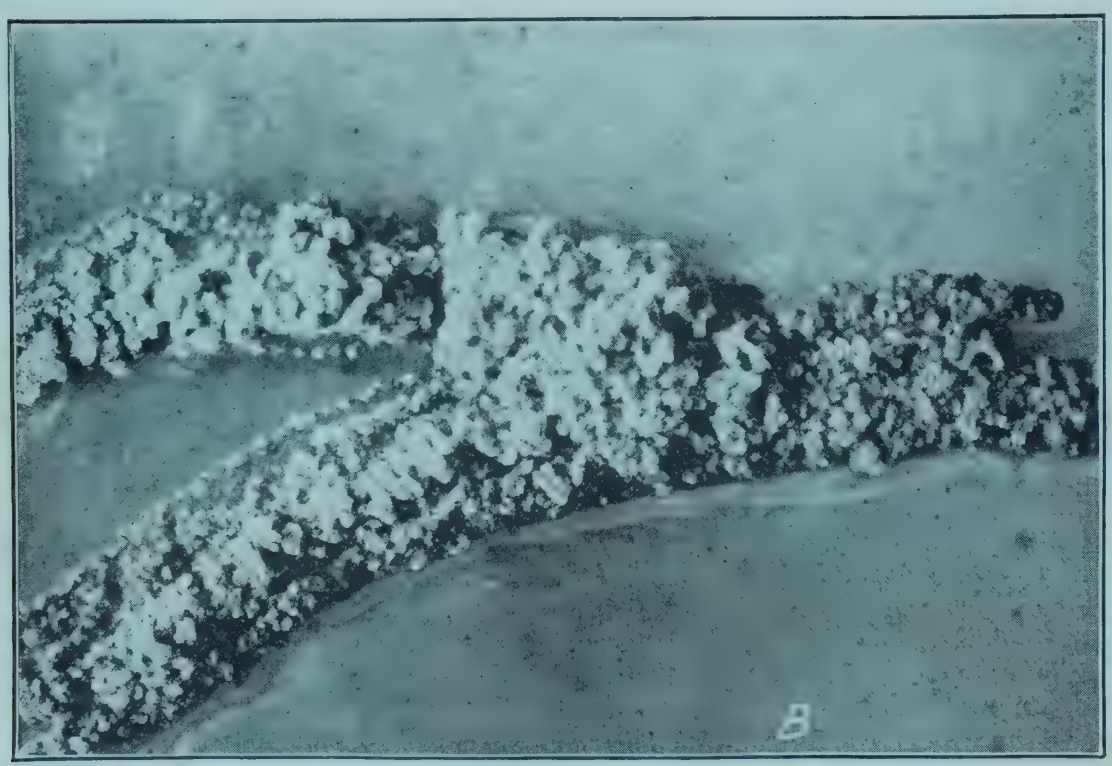
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PLATE 54

Sorosporella uvella:

A.—General appearance of the thallus when grown on Molisch's medium, showing brainlike convolutions and crateriform structure. Natural size.

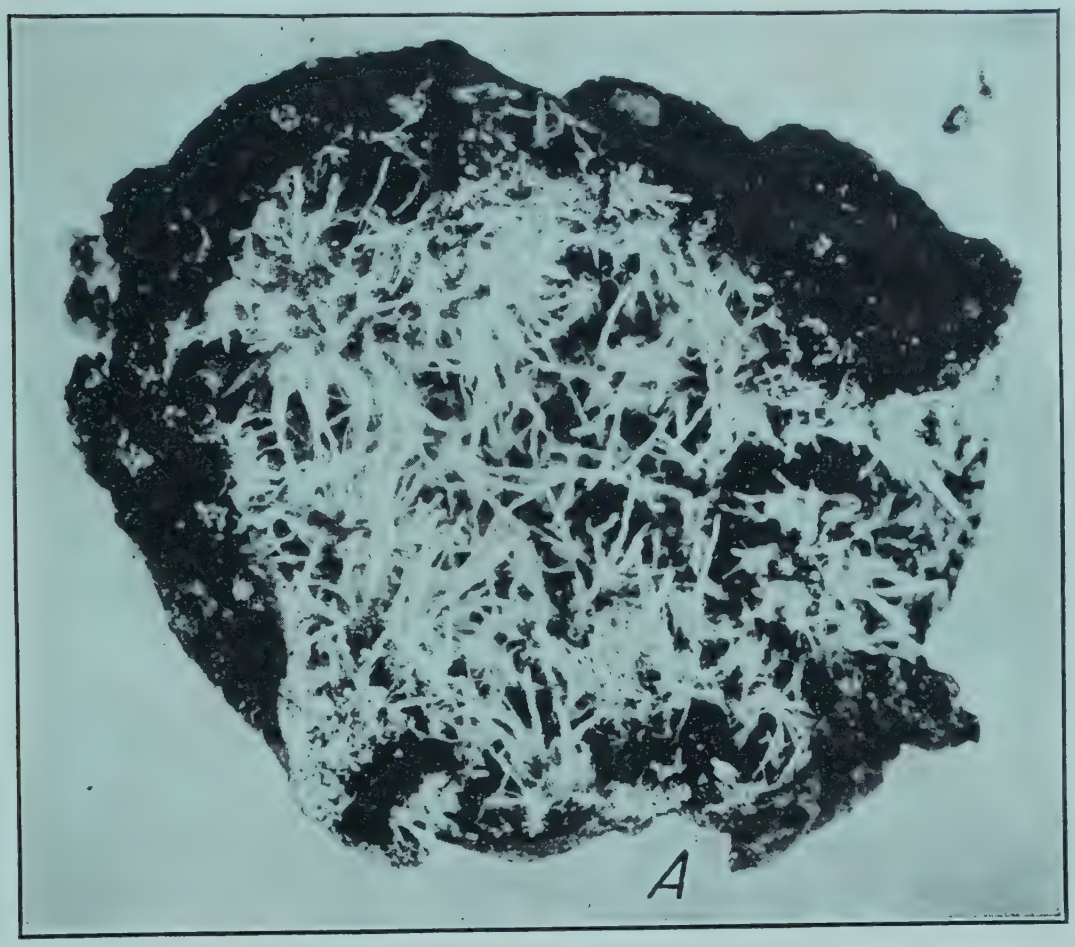
B.—Streak culture upon beerwort agar, showing shiny gelatinous-appearing, grape-like bunches of mature resting spores, some of which are germinating. × 5.

PLATE 55

Sorosporella uvella:

A.—Infected larva of *Feltia* sp. torn open, showing Isaria-like fascicles of conidiiferous hyphæ that sometimes develop when the resting spores are allowed to germinate in a moist chamber. × 10.

B.—Photomicrograph of a stained cross section of the heart, within the cavity of which numerous blastocysts are to be seen. × 125.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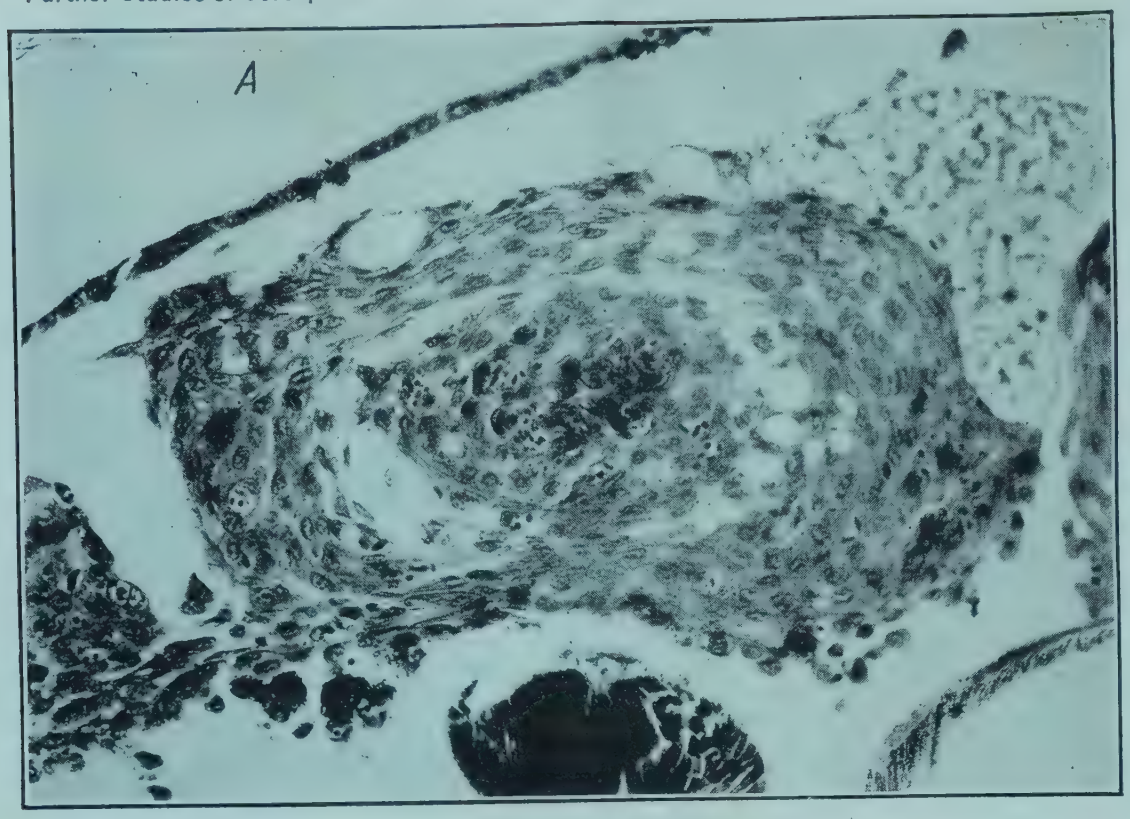
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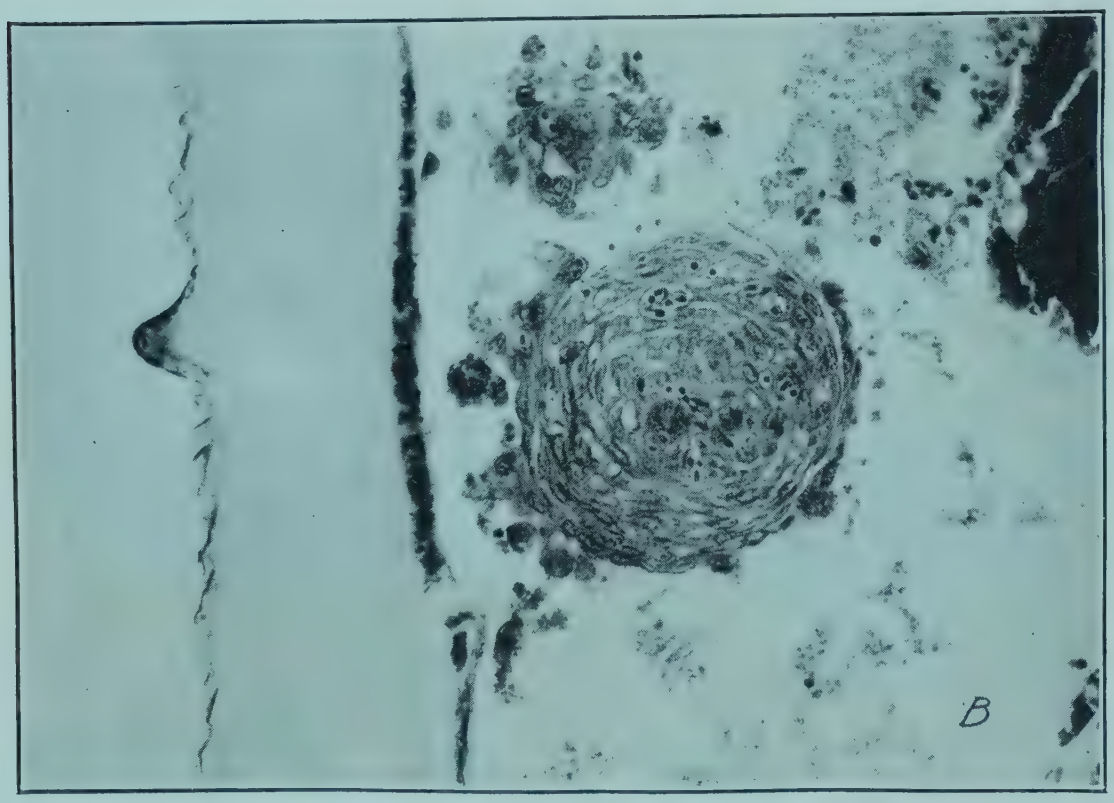
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PLATE 56

Sorosporella uvella:

A, B.—Photomicrographs of phagocytic complexes, showing blastocysts incorporated in the substance of the phagocytes. × 125.

WORK AND PARASITISM OF THE MEDITERRANEAN FRUIT FLY IN HAWAII DURING 1918

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The Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) after its introduction into Hawaii in 1910 soon became the most destructive fruit pest known in the history of the islands. A favorable climate, abundance of host fruits, and the absence of effective control measures allowed it to multiply so rapidly that it soon became established on all the important islands of the Hawaiian group, where it greatly retarded their horticultural development. Immediately after the discovery of the fly in Hawaii, its importance as an enemy was recognized; and the Federal and Territorial governments began to make exhaustive studies of its life history and habits and to experiment with different methods of control.

The use of natural enemies is the only control measure that has been to any great degree successful. Since 1913 the Hawaiian Board of Agriculture has introduced and successfully established in Hawaii four larval parasites of *Ceratitis capitata*—namely, *Opius humilis* Silvestri, *Diachasma tryoni* Cameron, *Diachasma fullawayi* Silvestri, and *Tetrastichus giffardianus* Silvestri. Every year since the establishment of these parasites, the Bureau of Entomology has gathered and published exact data on the degree of control exerted by them over the fruit fly, both as individual species and collectively (*1-4*).[1] This series of publications will be of value to entomologists who are interested in beneficial insects, by giving them definite information regarding the seasonal and yearly success achieved by each one of these four species. The taxpayer, who has paid the expenses connected with the introduction of these parasites, will find in these papers much definite information regarding the benefits he is deriving from his investment. This paper, therefore, is a continuation of these yearly records and gives the extent of parasitism during 1918, the amount of infestation by *C. capitata* for the same period, and, for purposes of comparison, general summaries of parasitism and infestation during 1916 and 1917, taken from literature already cited.

By reference to the data in Table I it will be seen that the infestation during 1918 of a number of host fruits is as great as it was during 1917, and in a few cases considerably greater. All these increases in infestation, with the exception of that in the mango (*Mangifera indica*), have taken

[1] Reference is made by number (italic) to "Literature cited," p. 446.

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place in the most preferred host fruits—that is, in fruits that consistently show high degrees of infestation. The great increase in the infestation of the mango may be accounted for by the fact that some varieties are much more susceptible to fruit-fly attack than others. In 1916 and 1917, infestation records were obtained from 1,317 and 648 fruits respectively, which were collected from a large number of mango trees of different varieties. Many of these varieties showed little or no infestation, and as a result there was a low average infestation for each of these two years. The scarcity of mangoes in 1918 made it impossible to obtain more than 85 fruits, which were of varieties preferred by the fruit fly. This accounts for the high average infestation of 24.4 larvæ per fruit. Those fruits, showing by their comparatively low average infestation that they were not especially preferred as hosts by the fruit fly, contained no more, and in a number of cases contained fewer, larvæ in 1918 than were found in 1917. This lack of increase in infestation of the less-favored host fruits confirms the conclusion drawn from the parasite records of 1917 (*4*)—that, although 50 per cent of the fruit-fly larvæ are destroyed by these parasites and other agencies, little relief is afforded the preferred host fruits, while much benefit is derived from the decreased infestation of the less susceptible host fruits.

This is shown in Tables I to III.

TABLE I.—*Extent of infestation of host fruits by larvæ of Ceratitis capitata in Hawaii during 1918*

Host fruit.	Number of fruits collected.	Number of *C. capitata* larvæ emerging.	Average number of larvæ per fruit.		
			1918.	1917.	1916.
Indian almond (*Terminalia catappa*)	25,558	252,067	9.9	8.0	9.5
Mango (*Mangifera indica*)	85	2,076	24.4	8.1	1.7
Coffee (*Coffea arabica*)	49,130	27,517	.6	.8	.5
Strawberry guava (*Psidium cattleianum*)	24,585	31,692	1.3	2.0	1.6
Black myrobalan (*Terminalia chebula*)	5,664	27,047	4.8	5.9	7.0
Peach (*Amygdalus persica*)	815	18,248	22.4	15.2	20.5
Satin-leaf (*Chrysophyllum olivaeforme*)	1,380	4,376	3.2	3.4	2.0
Rose-apple (*Eugenia jambos*)	1,302	8,568	6.6	8.8	5.5
French cherry (*Eugenia uniflora*)	13,558	13,026	1.0	1.0	.8
West Indian medlar (*Mimusops elengi*)	12,216	30,680	2.5	1.8	5.3
Kamani (*Calophyllum inophyllum*)	888	2,119	2.4	2.4	3.3
Yellow oleander (*Thevetia neriifolia*)	169	1,009	6.0	5.7	3.6
Carambola (*Averrhoa carambola*)	81	74	.9	.6	1.3
Chinese orange (*Citrus* sp.)	7,450	13,349	1.8	1.8	3.1
Guava (*Psidium guajava*)	3,481	29,542	8.5	4.5	6.8
Loquat (*Eriobotrya japonica*)	5,343	9,606	1.8	2.6	.....
Noronhia emarginata	289	323	1.1	.....	.....

Although Table I shows that the infestation of host fruits in general was as great in 1918 as it was in 1917, Table II indicates that parasitism of the larvæ developing in the majority of the abundant host fruits was

higher than in previous years. Notable among these hosts are the Indian almond (*Terminalia catappa*), coffee (*Coffea arabica*), strawberry guava (*Psidium cattleianum*), and French cherry (*Eugenia uniflora*). The most important of the abundant host fruits producing low percentages of parasitism are the mango, kamani (*Calophyllum inophyllum*), Chinese orange (*Citrus* sp.), and guava (*Psidium guajava*). The low parasitism of the larvæ developing in these latter, especially in the guava, large areas of which are growing in all parts of the islands, strongly indicates that these fruits are the source of supply of the large number of fruit flies which cause the continual high infestation of favored hosts.

TABLE II.—*Percentage of larval parasitism of Ceratitis capitata in Hawaii*[a]

Host fruit.	Month of collection in 1918.	Number of larvæ emerging during first 2 to 6 days.	Percentage of parasitism.				
			Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total.
Indian almond	February	568	1.8	2.1	.....	0.2	4.1
	March	2,695	37.3	10.7	0.3	4.5	53.0
	April	92	17.2	.....	.....	4.3	21.7
	May	2,134	34.3	36.1	.....	2.9	73.3
	June	8,707	17.7	59.8	.....	1.6	79.1
	July	5,125	2.6	63.2	.1	3.2	69.1
	August	2,746	.8	37.9	.4	5.5	44.6
	September	3,527	1.9	26.6	.4	15.4	44.3
	October	5,748	7.1	25.1	.2	11.0	43.4
	November	6,434	31.4	23.4	.1	13.7	68.6
	December	1,526	22.0	39.6	.3	11.7	73.6
Mango	July	283	2.1	10.6	.4	.7	13.8
Coffee	January	410	2.2	22.9	10.7	.....	35.8
	March	9	22.2	.....	11.1	.....	33.3
	April[b]	1,510	12.0	70.5	8.6	.....	91.1
	September	145	2.1	.....	39.3	.....	41.4
	October	681	3.9	8.3	27.6	.....	39.8
	November	877	16.2	39.1	4.4	.....	59.7
	December	37	5.4	29.7	10.8	.....	45.9
Strawberry guava	January	525	8.8	24.6	2.3	4.4	40.1
	February	186	9.7	19.3	20.4	.5	49.9
	March	593	8.8	6.6	10.8	1.7	27.9
	April	1,531	15.7	13.6	5.1	1.0	35.4
	May	272	33.5	21.0	1.1	4.0	59.6
	July	741	.1	62.1	1.2	2.5	65.9
	August	1,004	.5	45.1	6.9	21.4	73.9
	September	393	2.0	39.0	20.7	6.9	68.6
Black myrobalan	January	1,104	.3	1.2	1.3	.5	3.3
	February	2,348	.6	.5	.3	.1	1.5
	October	190	.....	.....	.....	.....	.....
	November	1,166	.7	2.1	.....	7.9	10.7
Peach	March	733	.....	.....	3.8	.5	4.3
	April	602	.3	2.3	3.7	.2	6.9
	May	581	2.2	5.0	1.0	.7	8.9
	June	307	15.3	9.1	.....	16.3	40.7

[a] The majority of the fruits listed in this table were collected about Honolulu at low elevations. Much of the coffee, however, was collected on the island of Hawaii and in the country districts of the island of Oahu and came from points 1,000 to 2,000 feet above sea level.

[b] The April collections of coffee came from the Kona district of the island of Hawaii.

TABLE II.—*Percentage of larval parasitism of Ceratitis capitata in Hawaii*—Continued

Host fruit.	Month of collection in 1918.	Number of larvæ emerging during first 2 to 6 days.	Percentage of parasitism.				
			Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total.
Rose-apple	May	186	4.3	9.1	.....	.....	13.4
	July	362	.8	54.4	.....	2.2	57.4
	August	836	.6	28.0	.....	1.0	29.6
	September	386	8.0	41.5	.....	5.7	55.2
Satin-leaf	January	534	20.4	3.7	5.8	.2	30.1
	February	123	18.7	7.3	8.1	3.2	37.3
French cherry	January	532	4.9	18.8	7.1	.2	31.0
	February	83	4.8	1.2	.....	.....	6.0
	March	42	2.4	.....	.....	2.4	4.8
	June	1,212	1.1	71.8	5.9	.6	79.4
	July	813	3.1	49.9	18.7	.9	72.6
	August	265	.4	46.4	38.8	4.5	90.1
	November	175	8.6	3.4	.....	.....	12.0
	December	86	20.9	7.0	.....	1.2	29.1
West Indian medlar	March	77	.....	.....	.....	.....	.....
	April	139	.....	.....	.....	.....	.....
	May	1,220	2.8	.....	.....	.4	3.2
	June	1,360	2.8	9.1	.1	.7	12.7
	July	212	.....	7.1	.....	1.4	8.5
Kamani	January	466	.....	.8	.....	.2	1.0
	February	42	.....	.....	.....	.....	.....
	March	120	.8	1.7	.8	.....	3.3
	April	14	7.1	.....	.....	.....	7.1
	July	85	.....	38.8	.....	.....	38.8
Yellow oleander	September	23	.....	13.1	.....	.....	13.1
	October	266	5.6	4.5	8.6	3.8	22.5
Carambola	January	15	.....	13.3	.....	13.3	26.6
	February	18	5.6	.....	.....	5.6	11.2
	November	7	.....	.....	.....	.....	.....
Chinese orange	January	577	2.2	1.7	.2	.5	4.6
	February	95	.....	6.3	.....	.....	6.3
	March	135	.7	11.9	.....	.....	12.6
	April	71	.....	.....	.....	.....	.....
	May	41	2.4	.....	.....	.....	2.4
Guava	February	136	9.6	9.6	.....	.7	19.9
	April	716	5.8	1.5	.4	.1	7.8
	May	1,422	6.0	9.8	.2	2.7	18.7
	June	2,802	2.2	27.4	1.3	11.8	42.7
	July	1,158	.8	18.9	1.2	9.8	30.7
Loquat	January	1,056	4.4	12.3	17.4	.8	34.9
Noronhia emarginata	July	44	.....	11.4	.....	.....	11.4

TABLE III.—*Total parasitism of all larvæ of Ceratitis capitata collected in Hawaii during 1918, arranged by months*

Month.	Number of larvæ.	Percentage of parasitism.						
		Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total for 1918.	Total for 1917.	Total for 1916.
January	5,219	4.8	9.6	6.2	0.8	21.4	59.0	6.98
February	3,600	2.3	2.5	1.6	.2	6.6	32.9	19.5
March	4,404	24.1	7.9	2.3	3.2	37.5	63.5	14.7
April	4,675	10.3	27.8	5.0	.4	43.5	43.3	37.64
May	5,854	16.5	17.3	.2	2.0	36.0	40.9	26.69
June	14,388	11.8	48.6	.8	3.7	64.9	36.1	27.81
July	8,827	2.0	52.2	2.1	3.6	59.9	51.0	18.52
August	4,850	.7	38.1	3.8	7.9	50.5	33.1	37.5
September	4,471	2.4	28.0	3.4	13.3	47.1	52.4	45.2
October	6,885	6.5	22.0	3.2	9.4	41.1	45.2	44.3
November	8,659	25.3	21.7	.5	11.2	58.7	72.3	44.3
December	1,648	21.5	37.7	.4	10.9	70.5	34.2	44.1
Average for 1918	63,480	12.4	34.6	2.6	6.2	55.8	.....	.....
Average for 1917	72,139	12.7	20.3	7.3	7.2	.....	47.5	.....
Average for 1916	83,304	17.2	13.3	2.1	.6	.....	.....	33.2

Previous publications (*1–4*) give data that show the consistent ascendancy of the parasite *Diachasma tryoni* over *Opius humilis* during the warmer months of the year and the predominance of *O. humilis* in the cooler months. This interchange in the effectiveness of these two parasites is due to the ability of *D. tryoni* to destroy *O. humilis* when both occur in the same host larva, coupled with the decreased activity of the former during the cooler months (*5*). Records of parasitism for 1916 and 1917 (*3–4*) show that *O. humilis* gained this predominance for five and three months, respectively; while during 1918, as shown by Table III, this species was able to gain the ascendancy for only two months—March and November. This yearly decrease in the effectiveness of *O. humilis* is directly due to the yearly increase in numbers of *D. tryoni*, which is shown by the average yearly parasitism records given at the bottom of Table III. The average parasitism by *D. tryoni* increased from 13.3 larvæ to each fruit in 1916 to 20.3 in 1917 and to 34.6 in 1918, while the average parasitism by *O. humilis* consistently decreased. Although the parasitism by both *D. fullawayi* and *Tetrastichus giffardianus* was less in 1918 than in 1917, the total percentage of parasitism for the last year, on account of the increased effectiveness of *D. tryoni*, had increased 8.3, making the total parasitism for 1918, 55.8 per cent of all the fruit-fly larvæ under observation.

The carefully recorded data concerning the activities of the Mediterranean fruit-fly parasites in Hawaii show that their value as destroyers of this pest has consistently increased each year since their introduction,

until in 1918 they caused the destruction of considerably more than half of all the fruit flies developing in fruits about Honolulu. This great decrease in the numbers of this pest has been of direct benefit to the people of Hawaii by greatly decreasing the infestation of the fruits less susceptible to fruit-fly attack, since this class contains the majority of fruits of commercial value. It has been of value also to the fruit growers of the mainland United States by greatly decreasing the danger of the introduction of the fruit fly there.

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CYANOGENESIS IN SUDAN GRASS: A MODIFICATION OF THE FRANCIS-CONNELL METHOD OF DETERMINING HYDROCYANIC ACID

By Paul Menaul, *Assistant Chemist*, and C. T. Dowell, *Chemist, Oklahoma Agricultural Experiment Station*

There have been a few cases reported from this and other States of the poisoning of cattle while pasturing on Sudan grass. Prof. H. D. Hughes of the Iowa State College was so kind as to send us information which he had obtained on the subject. It seems that no determinations of the hydrocyanic acid in Sudan grass have been made except those made by Francis[1], and for that reason the writers decided to determine the percentage of the acid present in the grass at several stages of its growth.

Our first sample was cut on June 16 when the grass was 15 inches high. Other samples were taken at intervals of one week thereafter until the grass was cut for hay. There had been plenty of rain, and the grass had grown very rapidly.

The method used for determining the acid was that employed by one of the writers[2] in determining the amount of the acid in kafir. The grass was cut fine, bruised thoroughly in an iron mortar, and then covered with water in a flask and kept at 40° C. for two hours. After this it was made more strongly acid with tartaric acid and distilled into 30 cc. of a 2 per cent solution of sodium hydroxid. The cyanid was precipitated as Prussian blue. After being burned in a muffle furnace the iron oxid was weighed, and from this weight the weight of hydrocyanic acid was calculated. One or two of the last determinations were made by the use of the Francis-Connell method[3] as modified by the writer. The results are shown in Table I.

From this table there is seen to be a decrease in the hydrocyanic acid with growth. This would be expected since similar results have been obtained with other varieties of sorghums. More of the acid is present in the leaves than in the remainder of the plant. This has been found by others to be true for other plants. A number of determinations which are not given in the table show that there is more hydrocyanic acid in the plant in the morning than in the afternoon. The writers

[1] Francis, C. K. poisoning of live stock while feeding on plants of the sorghum group. Okla. Agr. Exp, Sta. Circ. Inform. 38, 4 p. 1915.

[2] Dowell, C. T. cyanogenesis in andropogon sorghum. *In* Jour. Agr. Research, v. 16, no. 7, p. 175–180. 1919.

[3] Francis, C. K., and Connell, W. B. the colorimetric method for determining hydrocyanic acid in plants with special reference to kafir corn. *In* Jour. Amer. Chem. Soc., v. 35, no. 10, p. 1624–1628. 1913.

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are not aware that this observation has been made before. A comparison of the results obtained here with those given in the literature for kafir and other varieties of grain sorghums shows that Sudan grass contains about one-third as much hydrocyanic acid as do the grain sorghums.

TABLE I.—*Percentage of hydrocyanic acid in Sudan grass at different stages of growth*

Time of cutting.	Percentage of dry matter.	Percentage of hydrocyanic acid in leaves on dry basis.	Percentage of hydrocyanic acid in whole plant on dry basis.	Percentage of hydrocyanic acid in leaves on fresh basis.	Percentage of hydrocyanic acid in whole plant on fresh basis.	Remarks.
June 16	18.2	.....	0.0579	.....	0.0105	Height of plant, 15 inches. Plant was mostly leaves.
23	19.5	0.0465	.0274	0.0090	.0053	
30	23.7	.656	.0291	.0155	.0069	Rain of 0.53 inches on June 28.
July 7	31.0	.....	.0094	.....	.....	Grass cut between July 7 and 14.
14	31.0	.....	.....	.....	.0052	Grass cut in the morning.
					.0035	Grass cut in the afternoon.
31	22.0	.....	.....	.....	.0059	Second growth, 18 inches high.

MODIFICATION OF THE FRANCIS-CONNELL METHOD OF DETERMINING HYDROCYANIC ACID

Viehover and Johns[1] have criticized the Francis-Connell colorimetric method of determining hydrocyanic acid, pointing out (1) that the equilibrium of the reaction upon which the method is based is very sensitive to the presence of electrolytes and hence that the intensity of the color due to the ferric sulphocyanid is varied greatly by salts and (2) that a part of the sulphocyanic acid is volatilized in boiling the acid solution to remove the colloidal sulphur. Johnson[2] criticized the Viehover-Johns colorimetric method and used the Francis-Connell method after modifying it. The Viehover-Johns method is objectionable on account of the fact that the intensity of the color due to the Prussian blue varies with temperature and is changed by electrolytes. The Francis-Connell method would seem to be the better method if the objections pointed out by Viehover and Johns could be overcome. Johnson's modification of the Francis-Connell method makes the process too long, and the solutions used are more sensitive to electrolytes than are those used in the original method. The writers sought to modify the method and eliminate the objectionable features.

[1] VIEHOVER, Arno, and JOHNS, Carl O. ON THE DETERMINATION OF SMALL QUANTITIES OF HYDROCYANIC ACID. *In* Jour. Amer. Chem. Soc., v. 37, no. 3, p. 601–607. 1915.

[2] JOHNSON, Maxwell O. ON THE DETERMINATION OF SMALL QUANTITIES OF HYDROCYANIC ACID. *In* Jour. Amer. Chem. Soc., v. 38, no. 6, p. 1230–1235. 1916.

PREPARATION OF THE STANDARD SOLUTION

Ten cc. of a solution containing 5 mg. of hydrocyanic acid as potassium cyanid were placed in an evaporating dish, and 1 cc. of concentrated yellow ammonium sulphid and one drop of concentrated sodium hydroxid were added. This was slowly evaporated to dryness on a water bath by passing a current of air over the dish by means of an electric fan running at low speed. The residue was heated to 130° C. for five minutes then dissolved in 10 cc. of warm water acidified with dilute hydrochloric acid, two or three drops being added in excess. A 15 per cent solution of cadimum chlorid was added drop by drop until the sulphid ceased to form, and then a 10 per cent solution of ferric chlorid was added until the red color was permanent. This solution was then filtered through a moistened paper and 5 cc. of 10 per cent solution of ferric chlorid added to the filtrate. The volume was then made up to 100 cc.

PREPARATION OF THE UNKNOWN SOLUTION

One cc. of concentrated yellow ammonium sulphid was added to the distillate obtained by the process described above and then evaporated slowly on the water bath as in the preparation of the standard solution. The temperature was kept at about 70° C. The residue was treated as in the preparation of the standard solution. The standard and unknown solutions were then compared by means of a Bock-Benedict [1] colorimeter.

The method was tested by comparing a solution of potassium cyanid, which had been standardized by precipitation with silver nitrate, with a solution of potassium sulphocyanid, which had been standardized by the Volhard method. Two or three drops of dilute hydrochloric acid were added to each solution before the addition of the ferric chlorid.

The purpose of heating the residue to 130° C. was to prevent the colloidal sulphur from going back into solution. The maximum intensity of color was obtained with the concentration of ferric chlorid used by the writers. Johnson [2] used 2 cc. of a 1 per cent solution of ferric chlorid to the hundred and Francis-Connell [3] used 1 cc. of a 10 per cent solution. The writers found, as did Johnson, that the addition of hydrochloric acid to a solution containing but 2 cc. of a 1 per cent solution of ferric chlorid to the hundred increased the intensity of the color, and that the addition of potassium chlorid to such a solution had a bleaching effect. No change in the intensity of the color could be detected by either of the writers after the addition of the acid and salt to their solution. The results obtained by Johnson are what would be expected when dilute solutions are used.

[1] Bock, Joseph C., and Benedict, Stanley R. A new form of colorimeter. *In* Jour. Biol. Chem. v. 35, no. 2, p. 227–230, 3 pl. 1918.

[2] Johnson, Maxwell O., 1916. op. cit.

[3] Francis, C. K., and Connell, W. B., 1913. op. cit.

SUMMARY

(1) Sudan grass was found to contain about one-third as much hydrocyanic acid as is found in the grain sorghums. The quantity is greatest in the young plant and decreases rapidly as the plant matures.

(2) It was found that the colloidal sulphur formed by the Francis-Connell method could be removed by evaporating the solution to dryness and heating the residue to 130° C. for five minutes.

(3) When 5 cc. of a 10 per cent solution of ferric chlorid were used in 100 cc. of solution no observable change in intensity of color was produced by the addition of small amounts of hydrochloric acid and potassium chlorid either together or separately.

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EUROPEAN FRIT FLY IN NORTH AMERICA

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INTRODUCTION

The investigations reported in the following pages were made at La Fayette, Ind., except as otherwise indicated, in the years 1914–1916. They relate to the European frit fly, an insect which attacks both winter and spring wheat every year over the whole geographical range of the crop and at times has done considerable damage. This fly has received little study in this country on account of its minute size, the difficulty of carrying it through its life cycle in cages, and the confused and uncertain condition of its classification, which made it impossible to tell how many species were involved. The similarity of its attack to that of the Hessian fly has also, no doubt, in many cases prevented its recognition as a grain pest.

HISTORY AND SYNONYMY

In 1750 a paper was presented by Linnaeus (*13*),[1] the celebrated naturalist, to the Swedish Academy of Sciences, in which he described the injury caused to barley in Sweden by the larvæ of a small black fly which he found was destroying the immature kernels of the grain to a serious extent, in many heads eating out almost every kernel. Such light and worthless kernels the Swedes called "frits." The species was not given a scientific name until 1758, when the same writer (*14, p. 598*) described it as *Musca frit.* Fabricius (*7, p. 216*) in 1805 included the species under Oscinis, and it has generally been known as *Oscinis frit* since that date.

A century after the species had been described, it was found that the larvæ of the late fall brood winter as stem miners in winter grain, chiefly rye, in Europe, and that spring grain, especially rye and oats, is attacked in the same way by the spring brood. The summer brood was found to attack the kernels of oats more commonly than those of barley. These differences were for a long time supposed to indicate that several, at

[1] Reference is made by number (italic) to "Literature cited," pp. 472–473.

least two, species were involved; but finally a substantial agreement has been attained in the belief that the damage is the work of the single species, *Oscinis frit.*

In the United States the first biological observations on the species were made by H. Garman (*10*) in Kentucky in the fall of 1889, when he found the stems of young wheat infested. James Fletcher (*8; 9, p. 158*) made the same observation at Ottawa, Canada, at about the same time, and also reared the fly from larvæ in stems of several grasses. Both of these entomologists mention the insect as *Oscinis variabilis* Loew, a species described from North America.

In 1896 Lugger (*17*) reported the insect as *Oscinis variabilis* Loew, injuring spring wheat in Minnesota.

In 1898 Coquillett (*4*) reported various rearings of what he identified as *Oscinis carbonaria* Loew (*15, 16*) and *O. soror* Macquart. F. M. Webster (*21*) also reported rearing the same two species from various plants in 1903, his specimens having been identified by Coquillett.

Tucker (*19*) described a dark form from Colorado in 1908 as *Oscinis nigra*, without reference to its habits.

In 1912 a monograph on the Oscinidae (or Chloropidae, as he called them) of North America was published by Theodor Becker (*2*), of Liegnitz, Prussia, one of the most eminent specialists on this group. His work was based almost entirely on material furnished from this country by Prof. A. L. Melander, of Pullman, Wash., and the writer. Becker identified *Oscinis frit* from North America for the first time, with its variety or color form *O. pusilla* (see Meigen *18, v. 6, p. 157*, and *p. 160* for *Chlorops pusilla* and *C. frit*). What had been called *O. carbonaria* he made a synonym of *O. nitidissima*, a European species described by Meigen (*6, p. 388*) in 1838, which he regarded as distinct from *O. frit.*

Criddle (*6*) in 1913 discussed the larval habits in Manitoba, believing that he had detected three summer broods.

Starting with North American material identified by Becker as *Oscinis frit, O. pusilla*, and *O. nitidissima*, and with European material identified as *O. frit* by Prof. M. Bezzi and by Rev. Gabriel Strobl, the writer has made a study of the North American forms. This has extended to: (a) An examination of the type material of *O. variabilis, O. carbonaria, O. nigra*, and many other more or less related species; (b) a study of reared and collected material in most of the larger collections of the United States and Canada, including numerous rearings by members of the Bureau of Entomology staff and nearly 16,000 specimens of the species swept from vegetation in various parts of the United States and Canada; (c) the rearing of more than 300 specimens from several different food plants; and (d) a comparative study of the male genitalia in North American and European material, including some bred from oats in England.

This study has led to the conclusion that *Oscinis pusilla*, *O. nitidissima*, *O. carbonaria*, *O. variabilis*, *O. nigra* Tucker, and *O. soror* Macquart are all synonyms of *O. frit*. The multiplicity of names has arisen from the variability and wide distribution of the species. The long-continued confusion has existed mainly because the taxonomists since Linnaeus who have classified the adults have paid little or no attention to the habits, while the biological workers have been uninformed as to the characters used in classification and have applied whatever name was given them.

Typical *Musca frit* as defined by Linnaeus has the femora and tibiæ wholly black; *Oscinis nigra* Tucker is precisely this, as described from Denver, Colo. This extreme form apparently does not occur east of the Rocky Mountain region but is common westward. *O. frit* as recognized by Becker, however, includes eastern and western forms with a small amount of yellow on the tibiæ, at base and tip. When the yellow portions are more extensive, so that the front and middle tibiæ are merely ringed with black, the form *O. pusilla* is reached, of which *O. carbonaria* is an exact synonym. From this form *O. variabilis* differs only in having a more shining thorax and is the same as *O. nitidissima* of Europe, North American specimens having been so identified by Becker. All gradations from the somewhat shining dorsal surface of *O. frit* to the highly shining one of *O. nitidissima* can be readily found. Coquillett separated some specimens with shorter frontal triangle as *O. soror* of Macquart, but this is also a variable character; moreover, Macquart in describing it said nothing about the species having a short triangle.

NATURE OF INJURY

In the commonest form of injury minute maggots occur in young stems of wheat close to the ground. They are easily distinguished from the larvæ of the Hessian fly (*Phytophaga destructor* Say) from the fact that the larva is in the center of the stem and crawls actively when removed, whereas the Hessian-fly larva is between the bases of the leaves and is extremely inactive. The *Oscinis frit* larva often causes the central leaf to die and turn brown, those about it remaining green; this the Hessian fly larva never does.

DISTRIBUTION IN NORTH AMERICA

The region of greatest abundance of the frit fly in North America corresponds rather closely with that in which winter wheat is grown, from the Great Lakes to the Ohio River, and westward about as far as the Missouri. But outside this area it is often common locally from the Atlantic to the Pacific and from Ottawa, Canada, to the Gulf of Mexico. The fly occurs generally wherever grass is abundant and remains green for a considerable part of the year. So in the arid West it occurs in spots, along streams or in irrigated pastures, or in the higher altitudes where

the humidity is greater. Sweepings by the writer in 1917 gave numerous specimens at Pass Christian, Miss., and Lake Charles, La. A few were found at Marfa, Tex., Las Cruces, N. Mex., and Tucson, Ariz.; but none at Yuma, Ariz. Other species replace this one on grass at San Diego and Los Angeles, Calif., but it extends from San Francisco as far south as Santa Barbara. In Canada *Oscinis frit* occurs but sparingly in Manitoba, Saskatchewan, and Alberta but is much more numerous in the more southern latitude of Ontario and Quebec. No records are available for the extreme East. It has been reported from Juneau, Alaska, latitude about 58°. This is the farthest north of all existing North American records.

The accompanying map (fig. 1) has been dotted to indicate the distribution and approximately the abundance of the species, although in

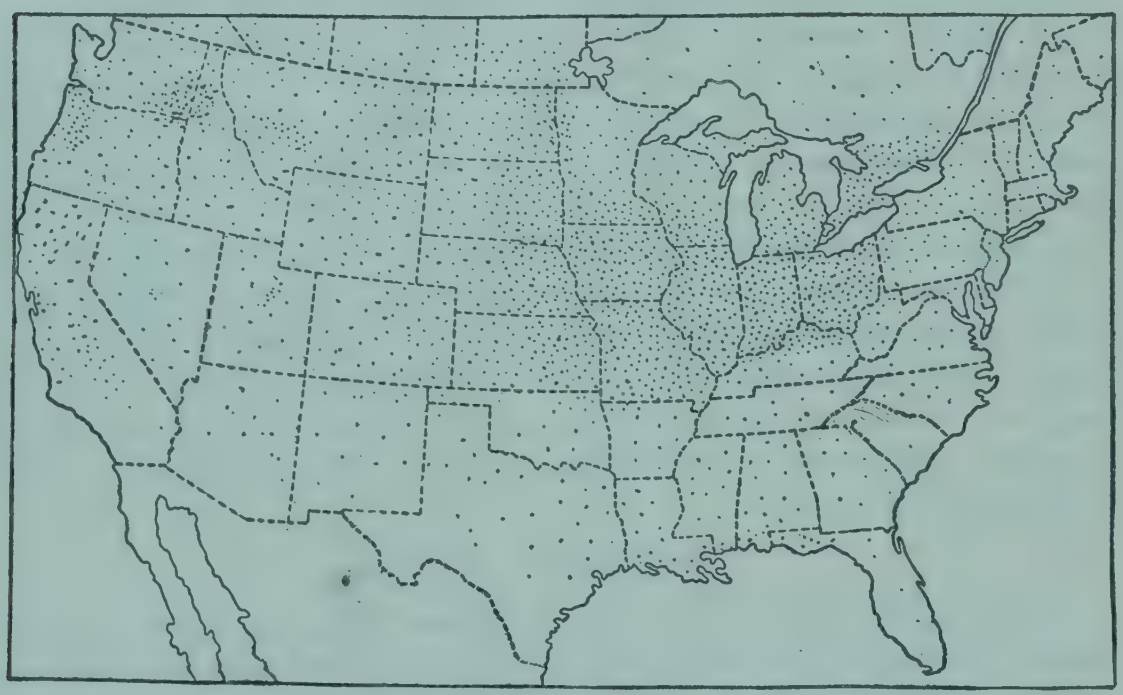

FIG. 1.—Map showing distribution of *Oscinis frit*.

many parts it is marked more from analogy than from definite information.

DESCRIPTION OF INSTARS

EGG

The eggs (fig. 2) are pure white, measuring 0.7 mm. in length and 0.178 mm. in greatest diameter, approximately straight on one side, the other more curved. The surface bears nearly 20 fine ridges, running lengthwise, which are occasionally broken.

NEWLY HATCHED LARVA

The larva (fig. 3) upon emerging from the egg measures 1.06 mm. in length and 0.14 mm. in greatest diameter. It is whitish and semitransparent in color, without head, rather pointed in front and truncate behind. A pair of very minute, soft, 2-jointed antennæ are present. The

only firm structures are the two mouth hooks and the frame to which they are attached. The hooks are clear reddish brown in color. The frame is black at their attachment and becomes gradually less chitinized and paler farther back, and more concealed by the mass of muscles surrounding it. Figure 3 shows one-half of this double structure. The hooks work on a pivot at the point marked *x* in the figure. The larva has 11 segments, the sutures between them, except the first three, bearing transverse rows of very fine teeth below, which extend up the sides in a narrowing series. The first segment is encircled by several rows of these minute teeth, evidently of use in entering crevices. At this stage there appear to be no anterior spiracles, the only ones being a pair at the posterior end; these are on raised protuberances bearing a circle of branched hairs standing at right angles to the axis, the opening into the air tube being on the inner side, not the tip, of the protuberance. From each spiracle a conspicuous air tube extends forward along the side of the body.

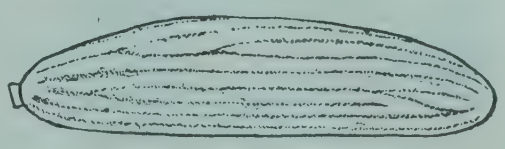

FIG. 2.—Egg of *Oscinis frit*. × 65.

FULL-GROWN LARVA

The full-grown larva (fig. 4) measures about 3 mm. in length and 0.4 mm. in greatest diameter. It is distinctly yellow in color on account of the accumulation of fat under the integument, for use during transformation. The antennæ and mouth hooks (fig. 5)) are relatively smaller than

FIG. 3.—Newly hatched larva of *Oscinis frit*. ×65. Antenna and mouth-hooks more enlarged, the latter showing fulcrum at *x*.

in the first stage. The mouth hooks are strongly curved, black, with several microscopic teeth on the under side. Anterior spiracles occur on the first segment, consisting of a protuberance bearing a half dozen soft lobes in a vertical row. The posterior spiracles appear to occupy a median position on their protuberances, with less distinct circles of hairs.

Intermediate larval stages were not made out.

PUPA

Like many Diptera, this species forms the pupa within the hardened larval integument, called the puparium. The pupa is never visible unless this shell-like covering is removed. It is white at first, becoming black

as the time of emergence draws near. It shows the organs of the adult in rough outline, the wings, however, being represented by very small rudiments.

PUPARIUM

This is at first yellow in color, turning to brown, and is usually opaque. It is 2.7 mm. in length by 0.9 mm. in breadth, bearing the minute larval spiracles anteriorly on each side of its tip and the posterior ones behind.

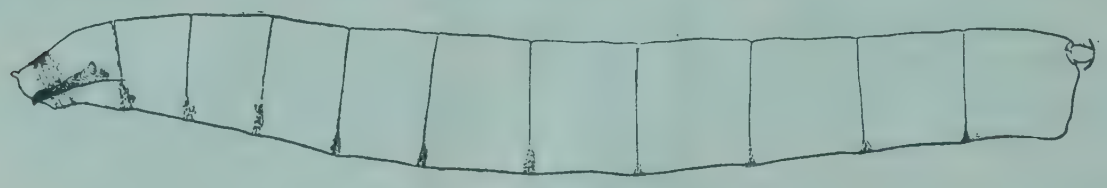

FIG. 4.—Full-grown larva of *Oscinis frit.* × 36.

Neither of these is functional, since they do not connect with the spiracles of the pupa. (Pl. 57, B.)

ADULT (PL. 57, A)

The following description of the adult has been drawn up with the aim of including all common variations.

MALE AND FEMALE

Length 1.1 to 2 mm. Head, thorax, and abdomen black. Front in well-matured specimens wider than one eye, usually a little narrower in specimens not completely hardened before killing. Frontal triangle shining black, reaching usually almost to the root of the antennæ, but shorter in many specimens, at shortest only a little over half the length of front; the remainder of the front outside the triangle is opaque black.

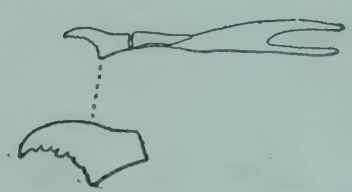

FIG. 5.—*Oscinis frit:* Mouth hooks of full-grown larva. More enlarged than in figure 4.

Face black, rather concave, its lower edge sometimes yellowish. Vertex bearing one outwardly directed, minute bristle near the corner of the eye; a minute, convergent pair of ocellars, and behind them a pair of also convergent ones on the occiput; a row of three or four small hairs along the inner edge of the eye in the opaque part of the front; a pair of hairs at the edge of the mouth also. Antennæ black, third joint rather large, round; the arista with very minute pubescence, beyond its basal fourth often lighter in color, rarely almost white when viewed against a dark background. Proboscis and palpi black. Bucca (side of head below eye) usually about one-sixth the height of the eye, but varying from one-fourth to one-tenth or less, the cause of this variation being usually the greater or less drawing up of the under part of the head in drying.

Thorax with length, breadth, and height about equal; dorsum subshining to shining, with minute dark hairs usually arranged in rows lengthwise. One pair of minute dorsocentral bristles before the scutellum, far apart; one supra-alar, two or three notopleural, one humeral, all small. Scutellum of ordinary form, neither flattened nor elongated. Pleuræ shining black, without bristles. Halteres yellow.

Abdomen black, subshining, rarely the first segment yellowish; the black color extending underneath to the soft part, which is usually paler. Male genitalia often protrude, showing a pair of distinct claspers curved backward, but these may be retracted and invisible. Abdomen of female pointed, ending in a minute pair of palpus-like organs, at tip of the telescopic, 3-jointed ovipositor when the latter is extended (fig. 6), but ordinarily so retracted as to be barely visible.

Legs of ordinary structure; coxæ and femora black, the trochanters and knees often yellowish; tibiæ rarely entirely black, usually paler at base and tip, the fore and middle tibiæ often wholly yellow, hind ones, however, always with at least a black ring. Tarsi yellow, darkened toward tips.

Wings subhyaline, sometimes a little brownish, varying moderately in width; costa extending to fourth vein; the costal segment between the tips of the first and second veins about 1½ times as long as the following one; fourth vein ending very slightly behind the apex; anal angle well developed.

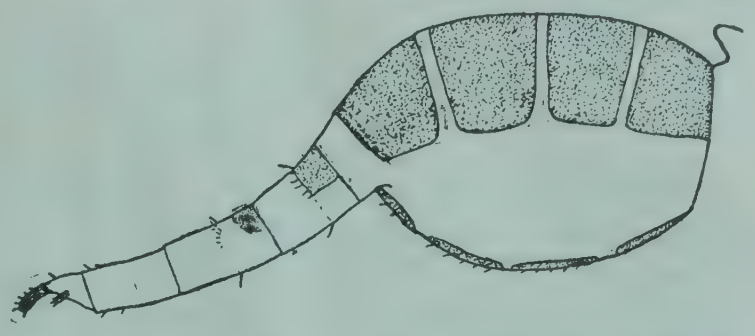

FIG. 6.—*Oscinis frit:* Female abdomen, distended with eggs and ovipositor protruded. (From alcoholic specimen, highly magnified.)

MALE GENITALIA

Since these organs in many insects throw a great deal of light on the limits of species, the genitalia of about 25 males were mounted for study after being boiled for from 5 to 10 minutes in 10 per cent caustic potash; among these were 5 specimens of the oat fly from Garforth, England, kindly furnished by Prof. T. H. Taylor, of Leeds University; others were from Missoula, Mont., Sioux City, Iowa, and La Fayette, Ind. No appreciable difference was found in any of these. The general features are shown in figure 7, drawn from a specimen taken at Missoula, Mont.

The fifth abdominal segment in the male is very small and normally retracted so as to be invisible. The sixth segment is also small, rather cup-shaped, open behind and below, and at least its posterior part is visible in life. It bears a symmetrical pair of long claspers below, curving backward and toward the middle line. The anus is situated at the middle of the segment behind; and on each side of this is a protruding, strongly chitinized lobe, which is produced forward inside both above and below; the upper arm keeps close to the side of the cavity and joins the produced upper margin of a curved plate with a thickened edge, which extends forward so as to form a trough, almost a tube, through the open anterior end of which the penis projects. This organ arises in the ventral part of the sixth segment in the median line and is supported in part from the ventral forward extension of the lobe beside the anus.

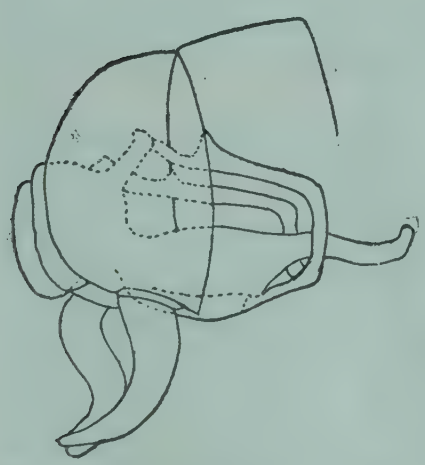

FIG. 7.—*Oscinis frit:* Male genitalia, highly magnified.

LIFE HISTORY

SUMMARY

At La Fayette, Ind., *Oscinis frit* winters in the larval stage in winter wheat. Following the emergence of this brood as adults in the spring, there are four summer broods.

DETAILED STUDIES

METHODS

On account of the minute size of the fly and its disinclination to lay eggs in cages, considerable difficulty was encountered in running a series of broods through the season, as well as in obtaining other details. The methods which finally proved successful are, therefore, believed to be of sufficient importance to justify a rather full description.

Wheat sown in the garden in September, 1915, became infested that fall. On March 30 and April 11, 1916, portions of it were dug up, placed in pans of earth, and covered with glass cylinders 8 inches in diameter with cheesecloth tops. Records of spring emergence were made from these. In removing the adults, advantage was taken of their natural inclination to go to light. The cage was opened in the house close to a south window on which the sun was shining. The flies, as soon as given their freedom, would fly toward the window, alight on a swiss curtain before it, and begin to walk upward. They were then easily secured in numbered homeopathic vials, in each of which a small drop of sirup and one of water had previously been placed. In these vials, tightly corked, adults were kept for some time, frequently 20 days or more, and in one instance 49, by changing the vial occasionally.

Breeding cages were prepared by placing earth known to be free from insects in 6-inch flowerpots, planting about 10 kernels of wheat close together in the middle of each, and covering with a lantern globe topped with cheesecloth. In seven or eight days under outdoor summer conditions the wheat was from 1 to 2 inches high, the proper stage for introducing the flies. A male and female were placed in the same vial and introduced into the cage by simply tipping up the lantern globe and setting the vial upright in the earth, removing the thumb from the mouth of the vial just as the globe was replaced. This method was not quite satisfactory, but only a few flies escaped. Their inclination to walk upward caused them to leave the vial at once if it was upright, but if it was horizontal they sometimes remained in it a long time, and even failed entirely to find their way out.

In the cage the flies were inactive most of the time, usually resting on the cloth at the top but occasionally visiting the plants, where they appeared to lick up a nutritious exudation. The addition of sirup did not lengthen their lives, and they soon died in cages without plants.

Eggs were obtained in a small proportion of cages, in from 20 to 40 per cent, as a rule. Many flies laid only 1 or 2 eggs, and the maximum was about 10. Since the normal number of eggs maturing in the ovaries of a female at one time is about 30, it is evident that the conditions were far from satisfactory; but they were the only ones under which any eggs were obtained.

On account of the small number of eggs secured, each successive generation in cages was much smaller than the preceding one. It is therefore necessary to start the season with a large supply of material to avoid running out before the end. With this species, though perhaps not with related forms, it is easy to stock up with adults of the first summer brood by placing considerable quantities of already infested garden winter wheat under cages as early as June 1. This wheat will yield adults in the desired numbers to stock cages for the second brood. Wheat taken from the fields is likely to be much less infested than that sown in gardens; the latter therefore should be used for cages.

Larvæ on hatching from eggs in cages immediately entered the young wheat stems and in most cases were allowed to develop there, the pot being watered and kept under observation to see when the adults made their appearance. For early larval stages, eggs were taken from leaves and placed in a vial, where they could be examined more frequently and closely than in the cage. In this way the newly hatched larvæ were obtained before they had fed. For later stages, larvæ were dissected out of wheat stems they had entered, placed on a fresh piece of young stem, and corked up in a vial. Larvæ so handled readily entered the second stem, which would keep fresh for four or five days; a second transfer would bring the larva to maturity—some, in fact, matured without it. The process of dissecting the larva out was performed under a low power of the binocular dissecting microscope, using a needle with a minute hook at tip and slitting the stem carefully. This is a simpler process than the description might indicate and was performed successfully hundreds of times with only a few mishaps. During the summer of 1915 as many as 100 vials containing transferred larvæ were under observation at once. The vigor and endurance of the larvæ are remarkable. When the stems were left too long in the vials and decayed, the larvæ almost always survived. In several cases they endured starvation for several days; one fasted a week and then when given the opportunity entered a wheat stem and in due time reached maturity. They do not survive drying up, however, but require a moist atmosphere. This is true also of the adults.

WINTER BROOD: DATES OF SPRING EMERGENCE

Records were kept in the spring of 1916 on four cages containing garden wheat that had wintered outdoors where it grew. The emergence is shown in Table I.

TABLE I.—*Emergence of winter brood of Oscinis frit*

Date of emergence.[a]	Cage No. 2, caged Mar. 30.	Cage No. 3, caged Mar. 30.	Cage No. 4, caged Apr. 11.	Cage No. 5, caged Apr. 11.	Total by days.
Apr. 26	1	0	0	0	1
29	0	0	0	0	0
30	0	0	1	5	6
May 3	5	3	0	10	18
6	2	0	2	9	13
9	0	1	1	4	6
15	1	0	1	3	5
19	0	0	0	0	0
25	0	0	1	0	1
31	0	0	0	0	0
June 12	0	0	0	0	0
Total by cages	9	4	6	31	50

[a] Dates on which no observations were made are not entered in the table.

From Table I it appears that emergence in 1916 extended over a period of 29 days (April 26 to May 25) and that 85 per cent of the flies emerged in 11 days, from April 30 to May 10. It would appear, however, that these dates were retarded by keeping the cages too much in the shade, since sweepings made outdoors at frequent intervals in early April gave the first adult for 1916 on April 13 and for 1915 on April 14.

SUMMER BROODS

Four complete summer broods were reared in 1916, as reported in Table II. Each cage was started at the time indicated by the cross, using flies that had emerged a few days before. Cages 23, 24, and 26 were exceptions, being stocked with garden wheat already heavily infested outdoors, This explains their large yield of flies. The wheat in cage 26 was taken up so late (June 16) that there was a possibility of its containing eggs of the second brood; and this probably accounts for the single fly which emerged in it on July 28, 16 days after the last preceding one. The negative observations for each cage are omitted up to the first positive one, after that both positive and negative are included up to the time that the use of the cage was discontinued. All cages that produced no positive results are omitted; they were more numerous than those included.

Flies of the first summer brood began to emerge on June 12 and continued to do so until July 13 (32 days), the heaviest emergence being from June 25 to 30.

Flies of the second summer brood began to emerge July 16 and continued to do so until July 26 (only 11 days). Flies emerged in 10 cages. July 21 gave the largest record.

Flies of the third summer brood emerged from August 10 to August 28 in 5 cages, a total, however, of only 21 flies.

TABLE II.—*Record of adults of Oscinis frit reared in cages at La Fayette, Ind., in the summer of 1916*

[The sign × indicates dates on which flies were introduced into cage. Observations were then made daily until the first fly emerged and thereafter only on dates indicated]

Date of observation.	Brood I.										Brood II.										Brood III.					Brood IV.	
	Cage No. —										Cage No. —										Cage No—					Cage No. —	
	11	13	14	15	19	20	21	23	24	26	27	28	29	31	36	39	43	44	45	52	73	74	75	82	84	87	92
May 6	×																										
9		×	×	×																							
15					×	×	×																				
31								×	×																		
June 12		2																									
13		1																									
14		1																									
15		0																									
16		1								×																	
18	2	1	1	1																							
19		0	0	0				1			×	×	×														
20		1		2																							
21	0			1					4																		
22	0	2	0	0					3					×													
23	2	2	2	1			1	5	4	2																	
26	0	6	0	0	1		8	26	41	1																	
27	0	1	0	0	0		0		21	2					×	×	×	×	×								
29	0						0	31	20	16										×							
July 2	0	1	0	0	0	1	0	9	0	7																	
5	0	0	0	0	0	0	0	10	3	13																	
6								1																			
7	0	0	0	0	0	0	0	1	7	8																	
10	0	0	0	0	0	0	0	0	1	7																	
13	0	0	0	0	0	0	0	1	1	4																	
16	0	0	0	0	0	0	0	0	0	0	1																
17								0	0	0	0	4	1														
19								0			0	0	3	1	4	1			1								
21									0	0	0	0	0	1	4	3	3	2	1	2	×	×	×	×			
24											0	1	0	0	0	1	0	0	2	0							
26											0	0	0	0	1	0	1	1	1	1							
28										1	0	0	0	0	0	0	0	0	0	0					×		
29										0																	
30											0	0	0	0	0	0	0	0	0	0							
Aug. 1											0	0	0	0	0	0	0	0	0	0							
3											0	0	0	0	0	0	0	0	0	0							
7											0	0	0	0	0	0	0	0	0	0							
10															0	0	0	0	0	0		3					
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12																					0	0	1				
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16																					0	0	0			×	
17																											×
19																					2	0	0				
21																					0	0	0	3			
23																					1	0	0	2	3		
24																					0	0	0	0	1		
26																					0	0	0	0	2		
28																					0	0	0	0	1		
31																					0	0	0	0	0		
Sept. 3																					0	0	0	0	0		
6																					0	0	0	0	0		
8																					0	0	0	0	0		
11																					0	0	0	0	0		
14																								0	0		
24																										1	1
25																										1	0
26																										0	2
27																										0	0
Oct. 2																										0	0
3																										0	1
4																										0	0
6																										0	0
10																										0	0
13																										0	0
15																										0	0

Only two flies of the fourth summer brood were obtained. These emerged in cage 87 on September 24 and 25. The small number was due to the rapid dwindling of the broods in cages, as explained under "Methods" p. 459. When a possible failure to get the fourth brood in lineal descent was anticipated, a supplementary series of cages was started, stocked with flies obtained by sweeping a bluegrass lawn. One of these, cage 92, gave adults, and they correspond very well with those of cage 87, the first fly appearing on the same date in both. Cage 92 gave two adults on September 26 and one, the last of the season, on October 3.

Cages started the last of September with flies swept from the lawn and with the few reared flies gave no results, no eggs being obtained. The weather was cool, and the flies were almost continuously dormant. Afterthought would suggest that in spring and fall the cages need a good deal of direct sunlight. No indications, however, of a fifth summer brood were observed. Flies emerging in September probably live longer than those of midsummer, having long dormant periods in cool weather; so they merely lay eggs on winter wheat in October for the winter brood.

The record here given covers too few flies, and those kept under too uniform conditions, to exclude the possibility that a portion of the representatives of this species, under natural conditions, might have a brood more or less. It does indicate, however, that four is the normal number of summer broods; and, allowing for the effect of a slight retardation in the cages in spring and fall, it is probable that five broods in the season will occur oftener than three.

LENGTH OF INSTARS

The number of days from adult to adult in each of the summer broods is shown by Table III.

In this table there were 8 individuals in brood II and 3 in brood III whose parents emerged two days apart, the intermediate day being taken as the date for both. One of these cases in brood III gave the minimum record of 22 days. The actual minimum period for the season was for the male of this pair. He was 21 days from emergence when his offspring emerged; but since his mate was 23 days old the number 22 was recorded as the average. The period covered in this case for the male was July 21 to August 11, in which there were two hot waves separated by a few somewhat cooler days—on the whole, an excessively hot period.

The table shows an average period from adult to adult in the first summer brood of 49.7 days for 35 individuals; for the second brood, 30.3 days for 41 individuals; for the third brood, 28.5 days for 21 individuals; and for the fourth brood, 45.5 days for 2 individuals.

TABLE III.—*Variation in the time of emergence of adults of Oscinis frit in each brood, with reference to the number of days from emergence of their parents*

Number of days since emergence of parents.	Brood number.				Total by days.
	I.	II.	III.	IV.	
22			1		1
24		1	4		5
25			1		
26		10			10
27		3			3
28		4	3		7
29		5	1		6
30		1	5		6
31		4	2		6
32		3	2		5
33		1	1		2
34			1		1
35		4			4
36		1			1
40		3			3
42		1			1
44	3				3
45				1	1
46	3			1	4
47	3				3
48	2				2
49	6				6
52	16				16
53	1				1
58	1				1
Total by broods	35	41	21	2	99
Average	49.7	30.3	28.5	45.5	

The period from adult to adult may be considered as consisting of: (a) The preoviposition period; (b) the egg instar; (c) larval instars; and (d) the pupal instar. Since the sum of these (adult to adult) varied from 21 to 58 days, naturally the components would vary accordingly—not, however, in strict proportion, because all periods become successively accelerated while approaching the heat climax of summer and are retarded after passing it. Making the best approximation possible, the records obtained seem to bear out the following division of the entire period:

PERCENTAGE OF TIME IN LIFE CYCLE

Preoviposition	14
Egg	11
Larva	50
Pupa	25
Total	100

It would be more logical to count this period from egg to egg, but oviposition is very difficult to observe and the data upon that subject are very meager. The emergence of the adult is the easiest point to note, and is the one on which the record is most complete.

HABITS AND FOOD PLANTS

As indicated in the introduction, the fly deposits its eggs on grains and grasses, usually on the very young and tender shoots, but sometimes upon or within the glumes just after heading. In the former case the larva enters the shoot and feeds downward in the middle; in the latter, it eats out the soft young kernel

Oviposition was not observed, although eggs were found that had been laid only an hour or two. In one instance, a female was seen to protrude the three terminal segments of her abdomen into a sort of ovipositor (see fig. 6, from an alcoholic specimen). With this she explored the crevices along the stem formed by overlapping of leaves but finally discontinued the operation without laying. Eggs were found both on the stem close to the ground and on the leaves, the latter, however, always within 3 or 4 inches of the ground. One or two lots were laid in crevices, but most of them were in plain sight. In about 5 days after the emergence of the female her eggs are fully developed and can be seen through the thin side walls of the abdomen. There are normally about 30 of equal size contained in the two ovaries. No indication was seen that other series might develop later, but such may be the case under outdoor conditions.

During the seasons of 1915 and 1916, sweepings were made on various grains and grasses, both by the writer and by a number of voluntary assistants,[1] to ascertain the distribution and preferences of the adult. Usually 200 sweeps with a 12-inch net were made. An examination of 240 lots was made in 1915 and of 310 lots in 1916, in order to sort out the various species of oscinids and related families. In both years *Oscinis frit* led all others in numbers, with totals of 4,677 and 11,235 out of grand totals of 23,416 and 40,187.

In the course of this work it soon became manifest that adults of *Oscinis frit* are rare on grain after it has begun to shoot up to head, or on grasses that are approaching maturity; but they are abundant on wheat and grass that is in an earlier stage, stooling or producing fresh shoots. Bluegrass lawns that are kept sprinkled and mowed yield large records of *O. frit* practically all through the season. Roadside bluegrass kept grazed yields large numbers before the dry weather of summer, but the records decrease rapidly at this time. Evidently the fly seeks grain or grass that is producing new shoots. They seem to be attracted by an exudation from the fresh epidermis, which they greedily lick, and not by the desire to lay their eggs on the plants. In no case was any appreciable number of specimens obtained from sweeping on dicotyledons, unless the stand was noticeably mixed with grass. The maximum record for 1915 was 365 flies in 200 sweeps with a 10-inch net on a blue-

[1] Among these should be mentioned Mr. Norman Criddle, Field Officer for Manitoba of the Dominion Entomological Branch, C. N. Ainslie, Sioux City, Iowa, and Dr. C. F. Adams, Atherton, Mo.

grass lawn at Elk Point, S. Dak., by C. N. Ainslie. This was on September 7.

This was exceeded five times in 1916, when the highest record was 486 flies in 200 sweeps with a 13½-inch net on bluegrass lawn at the Central Experiment Farms, Ottawa, Canada, by Mr. Germain Beaulieu, on August 17. Both of these records indicate a great concentration of the flies upon this food plant in late summer. However, an earlier record stood second in 1916, when Dr. C. F. Adams swept 401 flies in 200 sweeps of a 12-inch net at Atherton, Mo., on bluegrass lawn, on May 17.

INFESTATION OF WHEAT

Almost all the infestation observed by the writer has been upon wheat. Eggs are laid on fall wheat soon after it comes up, and the larvæ winter in the stems. Wheat was sown at weekly intervals from September 12 to October 17, and in November it was noted that the infestation was great in the earliest sowing and decreased regularly to the latest one or two sowings, in which none could be seen. In the spring the wheat is attacked by the first summer brood. Spring wheat is not a farm crop at La Fayette, but experimental sowings, especially the later ones, were heavily attacked.

A very characteristic symptom of infestation in young shoots of all kinds is the dying of the central leaf while the others around it remain green. The observer readily notices this when once his attention is directed to it. In the cooler and moister periods of the year, however, the insect may be abundant and yet only a few of the plants show this symptom. Since the larva does not usually cut the central leaf entirely off, in periods of low transpiration the leaf will still keep green for some time, whereas the same injury in hotter and dryer weather would kill the leaf at once. So the damage may be greater than it appears and can be calculated for the cool part of the year only by placing a known number of plants in a cage and counting the flies that emerge, every one of which may be considered to have destroyed a shoot.

At a meeting of Russian economic entomologists in Kiev in 1913, Mr. N. V. Kurdjumov (*1*) advanced the theory that the pruning off of some of the shoots of summer grain by *Oscinis frit* may do it good rather than harm. But the same author (*12*) in the same year mentions the insect as inflicting particularly severe injury upon spring grain. Since several other Russian entomologists have reported it to be doing serious injury, it is likely that its possibly useful character was mentioned in a qualified way.

Although in garden-sown wheat infestation occurred in a considerable percentage of the stems, the writer was never able to find in fields of winter wheat any appreciable infestation in late fall or early spring, such as was reported by Garman. For a while this was explained apparently by the fact that at these seasons of the year the characteristic

symptom of infestation (the dying of the central shoot) does not appear so quickly. In the spring of 1917 the point was further tested by transplanting wheat from fields to pots under cages, to compare it with rye and several grasses treated in the same way. Glass cylinders were placed over several grasses outdoors without transplanting, and several lots of wheat and grass were taken into the laboratory and all the stems slit up to find larvæ. The net result was that the fly was found to winter in timothy and meadow fescue, but not in wheat, rye, or several other grasses. The explanation which best harmonizes this result with other observed facts is that the insect has a rather wide range of habits and may concentrate upon any one of several food plants, just as it sometimes severely attacks the young, unripe grains while ordinarily it does not affect them.

INFESTATION OF RYE

A small percentage of infestation was obtained in rye sown in the garden rather late in spring. The European literature contains frequent references to *Oscinis frit* as a pest of rye, but the crop is so little raised in the United States that the insect heretofore has escaped notice in this connection.

INFESTATION OF BARLEY

Linnaeus (*13*) in his classic first paper on *Musca frit* described the infestation of barley kernels on an extensive scale, beyond anything that has been seen since. He estimated that one-fifth of the barley crop was annually destroyed by the insect. A few years later in another paper he reduced the estimate to one-tenth, indicating that further observation had not shown so great infestation as in the first instance. The insect also attacks barley stems in the spring, as indicated in European literature and confirmed by the writer.

INFESTATION OF OATS

The oat fly is a term used in England for *Oscinis frit*. Wilhelm (*24*) published a 40-page pamphlet in Germany on it, using the same name (die Haferfliege). In both countries it has often been noted mining the young shoots and destroying the ripening kernels. Westwood (*22*, *23*) reports a striking instance of infestation of oat kernels in England, attributing it to *O. atricilla* Zett. A farmer had thrashed 25 quarters of oats and stored them in a loft. The following account was written to Westwood by J. B. Yonge, Esq.

> A few days afterwards a stratum of flies was seen on top of the Oats, coming up among them, and passing away through the window. The stratum was about 4 feet long, 1 broad, and 3 inches thick, and being continually renewed from below as those above passed off, an immense number must have gone through during the four days it was observed.

This case compares very well with the immense infestation of barley kernels reported by Linnaeus in 1750 (*13*); no doubt both represent extreme instances of this sort of damage.

Professor T. H. Taylor, of the Department of Agriculture, Leeds University, England, has kindly given the following summary of his observations on the oat fly in England in a letter dated October 6, 1915.[1]

> The chief attack is made upon the oat crop. I have seen crops very seriously damaged by the pest. The attack that injures the oat plants most seriously is caused by the first brood appearing in early summer. These flies lay their eggs on the leaves of the young oat corn and the larvæ bore in the heart of the plant and destroy the stem. The plant thereupon tillers and produces a stunted bunch of young shoots which are practically worthless. The farmers call this condition of the oats "segging." The second brood of flies remains mostly—but perhaps not altogether—upon the oat crop, and the larvæ attack the *grains* between the glumes. These larvæ pupate *in situ* and the flies (third generation) migrate from the oats to wild grasses, upon which they spend the winter, pupating the following spring and giving rise to the first brood of frit-flies for the new season, thus completing the vicious circle. I have come across isolated examples of frit-fly attacking the stem of wheat and barley, but as I have paid very little attention to these outside attacks I can only say that they appeared to be due to the ordinary *frit*. I do not remember to have seen the grains between the glumes of either wheat or barley attacked.

When the writer's studies early revealed the fact that the American species has a marked distaste for the oat, several lines of investigation were carried out in order to test this relation as fully as possible.

(1) Wheat, rye, emmer, barley, and oats, sowed in rows in the garden in late spring, were infested in the order given, wheat much the worst, oats hardly at all. Oats sowed in the garden on August 25 were taken up and placed in a cage on September 20, when they were about 10 inches high and very thrifty. They had occupied a rather dense row about 25 feet long. In this cage only one specimen of *Oscinis frit* emerged, on October 16. A control cage of wheat sowed at the same time and caged at the same time yielded 68 adults of *O. frit*—12 on October 5, 20 on October 13, 8 on October 16, 20 on October 22, 3 on October 27, and 5 on November 1.

(2) Eight cages were started in which pairs of *Oscinis frit* were confined on young oat plants. Three eggs were laid in two cages, but no adults developed.

(3) In order to learn whether the American species is able to feed upon the oat stem at all, on September 10 and 13, 1915, 29 larvæ were dissected out of young wheat stems and placed on similar young oat stems in vials, as described under "Methods," p. 459, except that the food plant was changed. The results are shown in Table IV.

In most instances the larvæ which did not enter lived about a week, crawling actively on the glass much of the time.

[1] Professor Taylor disclaims any attempt to identify the species of the insect; specimens sent by him at the same time, however, seem indistinguishable from *Oscinis frit* as identified by Professor Bezzi and G. Strobl.

TABLE IV.—*Results of transferring larvæ of Oscinis frit from young wheat stems to young oat stems*

Larva No.	Result.
176	Died without entering oat stem.
177	Do.
178	Do.
179	Do.
180	Do.
186	Do.
187	Do.
188	Do.
189	Do.
190	Do.
199	Do.
200	Do.
201	Do.
202	Refused to enter oat stem, and after 7 days the nearly starved larva was placed in a vial with a wheat stem, which it entered and in due time emerged as a normal adult, Oct. 15.
203	Refused to enter oat stem, and after 10 days the nearly starved larva was placed in a vial with a wheat stem; it was however too weak to enter, and soon died.
204	Entered oat stem and fed; pupated normally; its emergence was not noted in the record.
205	Died without entering oat stem.
206	Do.
207	Do.
208	Entered and fed normally, and adult emerged Oct. 15.
209	Entered and fed, but left stem to wander on glass; however pupated and emerged Oct. 14.
210	Died without entering oat stem.
211	Do.
212	Entered and fed, but died without pupating.
213	Died without entering oat stem.
214	Do.
216	Larva was nearly full-grown and pupated without entering oat stem.
217	Died without entering oat stem.
218	Larva was nearly full-grown, and pupated apparently without feeding.

Thus it appears, disregarding larvæ 216 and 218, that out of 27 larvæ transferred, only 4 (204, 208, 209, and 212) accepted the oat as a food plant, and 1 of these did not reach pupation; 2 of the 4, however, emerged as adults and showed no differences from specimens reared entirely upon wheat.

A control series on the same dates, in which larvæ were dissected out of wheat stems and placed in vials on other wheat stems, gave the results recorded in Table V.

Of the 13 larvæ transferred in this test, only 3 died without entering the wheat stem, 7 went through their transformations normally, while the failure to get an emergence record for the remaining 3 probably is not due to the transfer. It may be presumed that in both series some larvæ suffered unnoticed injuries while they were being removed from the stems. The net result of the two series shows that larvæ of the American species, when they have once begun to feed in wheat, are very loath

to accept the oat, usually preferring starvation, whereas they can be transferred to new wheat stems with comparative ease.

TABLE V.—*Results of transferring larvæ of Oscinis frit from wheat stems to other wheat stems*

Larva No.	Result.
181	Fed normally, and adult emerged.
182	Do.
183	Do.
184	Fed and developed to pupation, but pupa became moldy and adult never emerged.
185	Died without entering.
191	Fed normally, and adult emerged.
192	Do.
193	Fed normally and developed to pupation, emergence not noted.
194	Died without entering.
195	Entered stem normally but was accidentally killed later while being transferred to fresh stem.
196	Fed normally, and adult emerged.
197	Died without entering.
198	Fed normally, and adult emerged.

(4) To determine whether specimens of *Oscinis frit* that had been reared in wheat would breed in green oat kernels, a bunch of newly headed oat plants was transplanted into a pan and arranged so that the heads only would project up through a slot in a horizontal wide board; an 8-inch glass cylinder, topped with cheesecloth, was placed over the heads, and the slot in the board was filled up and chinked with cotton batting. Into this cage with the oat heads 28 specimens of both sexes of the fly were introduced on July 2. On the next day 4 more pairs were introduced. None of the flies lived in this cage more than a week. On August 10 a single adult emerged, the only offspring of the 36 specimens confined.

(5) To test whether *Oscinis frit* or any other oscinid normally lives in oat kernels in the United States, it was planned to strip the green oats from 50 heads (estimated to be at least 1,000 kernels) and place them in a lantern-globe cage to see if any flies would emerge. The cooperation of economic entomologists was obtained, and in all 79 lots of 50 heads each were placed in cages. Twenty-two lots were from various places in northern Indiana; 2 from Madison, Wis.; 10 from Minnesota, sent by Professor Ruggles; 3 from South Dakota, sent by Mr. Severin; 7 from places in Montana, sent by Messrs. Cooley, J. R. Parker, and Larrimer; 4 from Washington, sent by Professor Melander; 5 from Utah, sent by Professor Titus; 13 from Colorado, sent by Director Gillette; 4 from Sioux City, Iowa, sent by C. N. Ainslie; and 9 lots received without data, but apparently from the West. The material was in various stages, but none fully ripe. It represented numerous varieties of oats, some being almost

pure wild oats. In only 2 lots did any flies emerge. One of these was taken at Manchester Siding, Ind., near Crawfordsville, on July 13 and yielded one specimen of *O. frit* on July 26. The other lot was taken on the edge of Crawfordsville the same day, and on July 26 it was found that two specimens of *O. frit* had emerged, together with one of *O. umbrosa* Loew and two of *Elachiptera nigriceps* Loew, a member of the same family.

A fair conclusion from the five lines of investigation would seem to be that *Oscinis frit*, as we have it in this country, does not normally feed upon the oat at all, but that occasional individuals, when compelled, are able to do so. This conclusion, in view of the marked preference for the oat manifested by *O. frit* in Europe, appeared to the writer to cast a strong doubt upon the identity of the American species; but after reviewing once more that phase of the subject he is of the opinion that there is no ground other than a physiological one for asserting that the species in North America is not *O. frit*. The case appears to resemble those mentioned by Dr. C. Gordon Hewitt (*11*) in his presidential address at the 1916 meeting of the American Association of Economic Entomologists, and others cited in the discussion of the address, in which strains of a species apparently arise which have a special adaptation to a certain food plant.

INFESTATION OF GRASSES

Much remains to be done in studying the relation of *Oscinis frit* to grasses. Only a few definite records of infestation exist, although most entomologists who have studied the insect assume that a considerable proportion of the flies, especially in middle and late summer, must breed in them. As already noted, sweepings made by the writer and by other entomologists who sent the material so obtained to him, show that from early summer onward the fly is much more abundant on bluegrass lawns than anywhere else. But sweepings on bluegrass that has begun to head or is in a later stage yield very few specimens, indicating that the presence of young shoots is the attraction. Sweepings on timothy in unmixed stand yield almost no specimens at any time, indicating that it is a plant unattractive to *O. frit*.

In 1915 five cages were prepared, each containing growing plants of wheat, bluegrass, and timothy. Several individuals of *Oscinis frit* of both sexes were placed in each. The only infestation that occurred was in wheat, from a stem of which a single maggot was taken and brought to maturity in a vial. From bluegrass sods placed in two cages no specimens of *O. frit* emerged, and several examinations of both stems and roots of the same grass gave no indications of infestation. *O. frit* has, however, been reared from this and other grasses, as the list of food plants will show.

KNOWN FOOD PLANTS

The following list of food plants includes those known for the United States and Canada. An asterisk (*) indicates that the fly was reared from this host by the writer. Numbers after authorities refer to the literature cited in this paper. The unpublished references are based on material identified by the writer.

*Wheat (stems). Garman (*10*), Fletcher (*8*), Webster (*21*), and Coquillett (*4*).
Wheat ("roots of wheat"). C. N. Ainslie, Moravia, Iowa (unpub.).
*Oats (stems). Webster (*21*), J. J. Davis, Sheldon, Ill. (unpub.).
*Oats (kernels).
*Barley.
*Emmer.
*Rye.
Corn (green cornstalks). Tucker (*20*).
*Timothy (*Phleum pratense*).
*Meadow fescue (*Festuca elatior*).
Kentucky bluegrass (*Poa pratensis*). Fletcher (*8*) and Webster (*18*).
Slender wheat-grass (*Agropyrum tenerum*). Fletcher (*8*).
Awned wheat-grass (*Agropyrum caninum*). Fletcher (*8*).
Quack grass (*Agropyrum repens*). Fletcher (*8*).
Rye grass (*Elymus canadensis*). Fletcher (*8*), and C. N. Ainslie, Elk Point, S. Dak. (unpub.).
Slough grass (*Spartina michauxiana*). C. N. Ainslie, Elk Point, S. Dak. (unpub.).
Barnyard grass (*Echinochloa crusgalli*). Webster (*21*) and C. N. Ainslie, Elk Point, S. Dak. (unpub.).
Low love-grass (*Eragrostis minor*). Webster (*21*).
Sedge (*Cyperus strigosus*). A. F. Satterthwait, La Fayette, Ind. (unpub.).
Cucumber roots. Webster (*21*).
Strawberry. Webster (*21*).
Ironweed (*Vernonia noveboracensis*). Wintering in seed capsules, Webster (*21*).

The last three records are the only ones on dicotyledons. It should be noted that the determinations were made at a time when the species of Oscinis were but little known, therefore they may be errors of identification or of observation.

PARASITES

Webster (*21, p. 56*) mentions having reared *Cyrtogaster occidentalis* Ashm. from either *Oscinis carbonaria*, *O. soror*, or *O. umbrosa* Loew, in Indiana. His uncertainty illustrates a common difficulty in rearing parasites from these forms. When material is taken from garden or field and placed in a cage to get the parasites, it is likely to contain the Hessianfly, Isosoma, Meromyza, Elachiptera, and several species of Oscinis. Although *Oscinis frit* may predominate, it is impossible to say positively that it was the host of the parasites. These are usually abundant. Even to isolate selected larvæ would not entirely obviate the difficulty, since at present no way is known to distinguish those of several species of oscinids. Parasitized larvæ would yield no adults, so there could not be a positive determination. Where cages are started by introducing adults on young wheat plants grown under cover, of course no parasitism is

possible; and this was the principal method used by the writer. Webster says:

Rhyssalus oscinidis Ashm. is parasitic on a species of Oscinis larvæ mining leaves of plantain at Washington;

but the miner referred to is now known to be *Agromyza melampyga* Loew, not Oscinis.

At present it can only be said that *Oscinis frit* appears to be freely parasitized by minute Hymenoptera, but observations have not as yet excluded all doubt in any case.

REMEDIES

The similarity of this insect's attack upon wheat to that of the Hessian fly indicates that a solution of the one trouble may carry the other with it. Unfortunately, the Hessian fly, although it has received a vast amount of attention, continues to inflict serious loss upon agriculture.

As far back as 1777, Bierkander (*3*) made recommendations for the control of *Oscinis frit* by changing the methods of tillage, and down to the present this is the only direction in which a lessening of its injury seems practicable.

Wheat sown early in the fall is more infested than that sown later, so the recommendation of late sowing to escape the Hessian fly will be equally applicable for *Oscinis frit*, but with this difference, that with the Hessian fly the possibility of infestation entirely ceases at a certain date, but with *O. frit* the chances decrease regularly until cold weather.

Wheat sown in the late spring is more infested than that sown early.

Continuous cropping in wheat appears to make no difference with the fly, which migrates freely for considerable distances.

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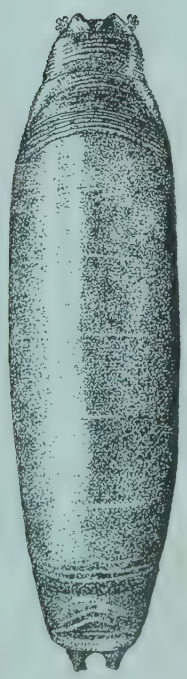
PLATE 57

Oscinis frit:

A.—Adult. × 35.
B.—Puparium. × 24.

A



B

LEPIDOPTERA AT LIGHT TRAPS

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INTRODUCTION

In the summer of 1916 extensive observations were made at the Hagerstown (Md.) field station of the Bureau of Entomology for the purpose of obtaining additional information concerning the relative proportions of the sexes of moths taken at light traps. The results of these observations have been stated in a paper recently published.[1]

While the observations of 1916 were being carried on, it was hoped that opportunity would be found in the summer of 1917 for securing more minute data as to the night-flying habits of Lepidoptera. Various circumstances contributed to the defeat of the project in 1917, and in the summer of 1918 it was conducted under conditions even more restricted than in 1916.

It was impossible to devote every night to the work, and the compromise schedule of two nights each week was unavoidably interrupted by the absence of the writer on field duty and by the moving of the laboratory.

The work was carried on during the period from May 14 to September 13, so the proposed schedule of two nights each week would have embraced 36 observations. On account of the interruptions mentioned, only 28 nights were given to the work.

DESCRIPTION OF TRAP

The attracting light, with accessory cone of tin, was described in the paper to which reference has been made. The trap devised by the writer is illustrated in figure 1.

The trap was 12 by 14 inches at the base and 20 inches high, constructed of heavy galvanized iron. An opening 9 by 10 inches afforded access to the interior and was closed by a door 10 by 11 inches, the overlapping half inch on each margin being covered by strips of heavy felt cemented to the trap, so that the door was practically air-tight. The galvanized iron thimble supporting the glass tube which conveyed the acid to the cyanid was lined with felt, and the opening in the top to receive the cone was provided with a felt gasket. A ball of absorbent cotton tied in muslin and dropped into the cone further retarded the escape of the hydrocyanic-acid gas. A glass vessel with rounded bottom

[1] TURNER, W. B. FEMALE LEPIDOPTERA AT LIGHT TRAPS. *In* Jour. Agr. Research, v. 14, no. 3, p. 135–149. 1918. Literature cited, p. 148–149.

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held the charge of sodium cyanid and was provided with a wire-cloth cover having at its center a square of wood bored to receive the stem of a small glass funnel. About five minutes were required to place the plug of cotton, pour the 25 per cent sulphuric acid into the glass tube, remove the dead moths, introduce a fresh charge of cyanid, and remove the tampon from the cone.

The trap was crated so that the door could be wedged tightly and the trap be made easy to handle.

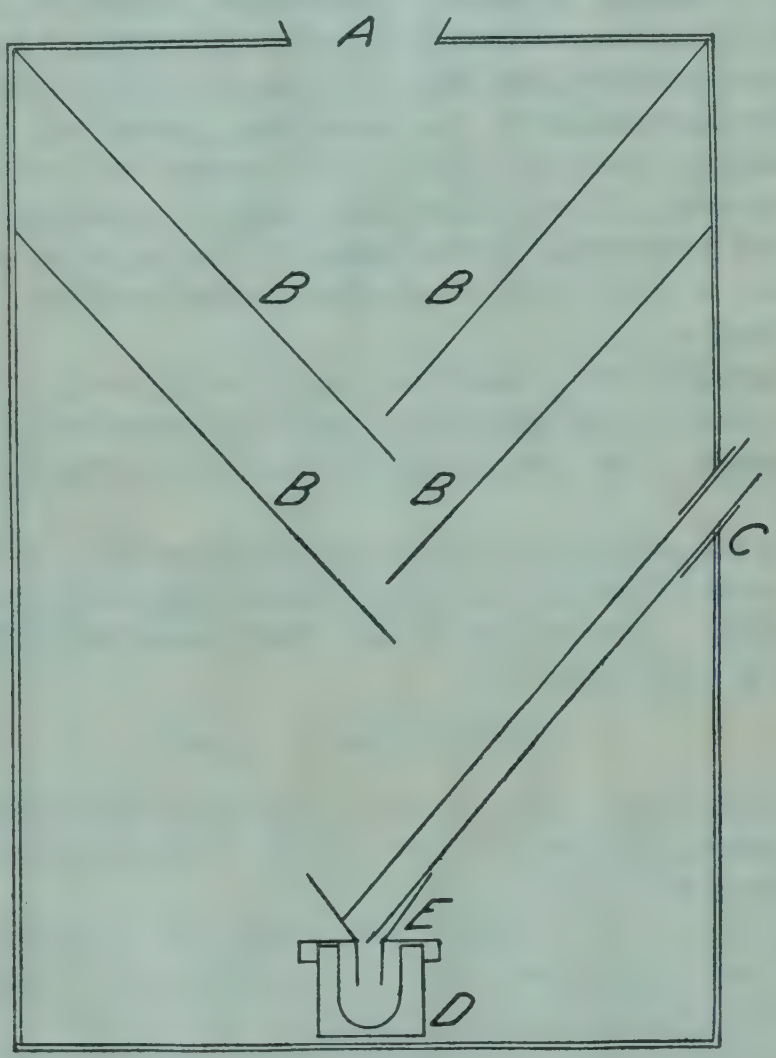

FIG. 1.—Light trap: A, opening for cone; BBBB, plates of glass; C, glass tube to convey acid; D, glass jar to hold cyanid; E, glass funnel.

RESULTS OF EXPERIMENTS

As has been stated, collections were made on 28 full nights between May 14 and September 13. The total of 3,152 moths recorded for that period embraces sixty-odd species. Of this total, 2,200, or 69.8 per cent, are males; and 952, or 30.2 per cent, are females. Table I gives an itemized account of the species, the numbers taken of each sex, and the percentage of males and females. In two species, *Noctua c-nigrum* and *Euparthenos nubilis*, the two sexes are equally represented; and of those species of which at least five individuals were taken, three show a preponderance of females.

TABLE I.—*Number and percentage of males and females of various species of Lepidoptera taken at a light trap, Hagerstown, Md., 1918*

Species.	Number of males.	Number of females.	Number of males and females.	Percentage of males.	Percentage of females.
Pholus pandorus	1	.....	1	100	.....
Phlegethontius 5-maculata	3	5	8	37.5	62.5
Darapsa myron	.....	1	1	.....	100
Darapsa pholus	1	.....	1	100	.....
Calasymbolus myops	1	.....	1	100	.....
Automeris io	1	.....	1	100	.....
Anisota rubicunda	2	.....	2	100	.....
Hypoprepia miniata	2	1	3	66.7	33.3
Utetheisa bella	.....	1	1	.....	100
Ecpantheria deflorata	.....	1	1	.....	100
Estigmene acraea	17	5	22	77.3	22.7
Hyphantria textor	118	13	131	90	10
Isia isabella	26	11	37	70	30
Diacrisia virginica	43	3	46	93.5	6.5
Apantesis virgo	2	.....	2	100	.....
Apantesis persephone	.....	1	1	.....	100
Apantesis vittata	174	20	194	90	10
Euchaetias egle	7	.....	7	100	.....
Pareuchaetes tenera	1	2	3	33.3	66.7
Halisidota tessellaris	166	62	228	72.8	27.2
Alypia octomaculata	.....	1	1	.....	100
Arsilonche albovenosa	3	2	5	60	40
Polia renigera	45	58	103	43.7	56.3
Dipterygia scabriuscula	10	2	12	83.3	16.7
Prodenia commelinae	9	3	12	75	25
Prodenia ornithogalli	16	12	28	59	41
Agrotis ypsilon	55	21	76	72.4	27.6
Noctua c-nigrum	157	154	311	50.5	49.5
Feltia spp	288	123	351	65	35
Mamestra meditata	8	2	10	80	20
Mamestra picta	5	2	7	71.4	28.6
Mamestra adjuncta	5	2	7	71.4	28.6
Cirphis unipuncta	34	21	55	62	38
Cirphis phragmitidicola	267	97	364	73.3	26.6
Meliana diffusa	104	63	167	62.3	37.7
Papaipema nitela	2	.....	2	100	.....
Pyrrhia umbra	2	.....	2	100	.....
Cosmia paleacea	3	1	4	75	25
Rhodophora gaurae	1	.....	1	100	.....
Schinia marginata	9	17	26	35	65
Autographa biloba	5	1	6	83.3	16.7
Autographa brassicae	89	58	147	60.5	39.5
Autographa simplex	49	20	69	71	29
Chamyris cerintha	1	.....	1	100	.....
Tarache aprica	6	1	7	85.7	14.3
Caenurgia erechtea	301	94	395	76.2	23.8
Caenurgia crassiuscula	122	37	159	76.7	23.4
Catocala similis	.....	1	1	.....	100
Euparthenos nubilis	3	3	6	50	50
Ypsia undularis	6	4	10	60	40
Datana ministra	22	5	27	80	20
Datana perspicua	2	.....	2	100	.....
Nadata gibbosa	1	.....	1	100	.....
Harpyia borealis	.....	1	1	.....	100
Cleora pampinaria	2	2	.....	100	.....
Xanthotype crocataria	1	.....	1	100	.....
Euclaena serrata	1	.....	1	100	.....
Azelina ancetaria	40	7	47	85	15

TABLE I.—*Number and percentage of males and females of various species of Lepidoptera taken at a light trap, Hagerstown, Md., 1918*—Continued

Species.	Number of males.	Number of females.	Number of males and females.	Percentage of males.	Percentage of females.
Tetracis crocallata	.....	1	1	.....	100
Harrisina americana	16	.....	16	100	.....
Atteva aurea	5	12	17	30	70
Total	[a] 2,200	[b] 952	3,152	.....	.....

[a] 69.8 per cent of total number of moths. [b] 30.2 per cent of total number of moths.

Table II gives the percentage of gravid females and shows that of 952 moths dissected, 736, or 77.3 per cent, were gravid, these constituting 23.35 per cent of all moths captured. Of the 11 genera of arctiids represented, all the females of 9 genera were gravid. Among the noctuids, gravid females made up 100 per cent in 8 genera.

TABLE II.—*Number and percentage of gravid female Lepidoptera taken at a light trap, Hagerstown, Md., 1918*

Species.	Number taken.	Number spent.	Number gravid.	Percentage gravid.
Phlegethontius 5-maculata	5	.....	5	100
Darapsa myron	1	.....	1	100
Hypoprepia miniata	1	.....	1	100
Utetheisa bella	1	.....	1	100
Ecpantheria deflorata	1	1	.....	.....
Estigmene acraea	5	.....	5	100
Hyphantria textor	13	.....	13	100
Isia isabella	11	1	10	90
Diacrisia virginica	3	.....	3	100
Apantesis persephone	1	1	.....	.....
Apantesis vittata	20	.....	20	100
Pareuchaetes tenera	2	.....	2	100
Halisidota tessellaris	62	12	50	80.6
Alypia octomaculata	1	.....	1	100
Arsilonche albovenosa	2	.....	2	100
Polia renigera	58	12	46	80
Dipterygia scabriuscula	2	.....	2	100
Prodenia commelinae	3	1	2	67
Prodenia ornithogalli	12	6	6	50
Agrotis ypsilon	21	6	15	71.4
Noctua c-nigrum	154	21	133	82.5
Feltia spp	123	44	79	64.2
Mamestra meditata	2	.....	2	100
Mamestra picta	2	.....	2	100
Mamestra adjuncta	2	.....	2	100
Cirphis unipuncta	21	8	13	62
Cirphis phragmitidicola	97	13	84	86.6
Meliana diffusa	63	20	43	68
Cosmia paleacea	1	1	.....	.....
Schinia marginata	17	3	14	82.4
Autographa biloba	1	1	.....	.....
Autographa brassicae	58	17	41	70
Autographa simplex	20	2	18	90

TABLE II.—*Number and percentage of gravid female Lepidoptera taken at a light trap, Hagerstown, Md., 1918*—Continued

Species.	Number taken.	Number spent.	Number gravid.	Percentage gravid.
Tarache aprica	1	.....	1	100
Caenurgia erechtea	94	31	63	67
Caenurgia crassiuscula	37	10	27	73
Catocala similis	1	1	.....	.....
Euparthenos nubilis	3	1	2	67
Ypsia undularis	4	.....	4	100
Harpyia borealis	1	.....	1	100
Datana ministra	5	.....	5	100
Azelina ancetaria	7	1	6	85.9
Tetracis crocallata	1	.....	1	100
Atteva aurea	12	2	10	83.3
Total	952	216	[a] 736	.....

[a] 77.3 per cent of total number of females.

TABLE III.—*Meteorological data, arranged by dates and hours of collections*

Date.	Temperature.		Humidity.		Time of flight.	Number of males and females.	Males.		Females.		Remarks.
	Max.	Min.	Max.	Min.			Number.	Per cent.	Number.	Per cent.	
1918.	°F.	°F.	P. ct.	P. ct.							
May 14–15	.....	.....	.....	.....	7 p. m.–1 a. m.	62	47	75.8	15	24.2	Electric current failed 1.05 a. m.
17–18	.....	.....	.....	.....	8 p. m.–4 a. m.	59	48	81.3	11	18.7	
21–22	.....	.....	.....	.....	7 p. m.–4 a. m.	60	55	91.7	5	8.3	Heavy fog 12 p. m. to 4 a. m.
24–25	.....	.....	.....	.....	.....do.....	24	16	66.6	8	33.4	Drizzling rain during entire period.
28–29	.....	.....	.....	.....	7 p. m.–12 p. m.	85	58	68.2	27	31.8	Material taken between 12 p. m. and 4 a. m. destroyed.
May 31 / June 1	88	72	78	70	7 p. m.–1 a. m.	512	366	71.5	146	28.5	
June 14–15	70	55	95	85	7 p. m.–4 a. m.	162	132	81.4	30	18.5	Heavy fog 2 a. m. to 4 a. m.
18–19	73	58	78	60	.....do.....	262	202	77.1	60	22.9	
21–22	63	54	88	87	4 p. m.–4 a. m.	35	25	71.4	10	28.6	Rain 7 p. m. to 3 a. m.
July 9–10	.....	.....	.....	.....	8 p. m.–4 a. m.	47	34	72.3	13	27.7	
12–13	.....	.....	.....	.....	.....do.....	52	35	67.3	17	32.7	
16–17	.....	.....	.....	.....	.....do.....	81	62	76.5	19	23.5	
19–20	.....	.....	.....	.....	.....do.....	63	50	80	13	20	
23–24	.....	.....	.....	.....	.....do.....	29	22	76	7	24	
26–27	85	73	89	65	.....do.....	40	36	90	4	10	
30–31	.....	.....	.....	.....	.....do.....	48	40	83.3	8	16.7	
Aug. 2–3	83	70	93	74	.....do.....	73	44	60	29	40	
6–7	98	95	89	87	.....do.....	232	151	65	81	35	
9–10	80	74	83	74	.....do.....	177	125	70.6	52	29.4	
13–14	75	71	85	83	.....do.....	101	59	58.4	42	41.6	
20–21	76	55	86	75	.....do.....	83	54	65	29	35	
23–24	90	75	81	70	.....po.....	88	47	53.4	41	46.6	
27–28	75	70	90	85	.....do.....	157	94	60	63	40	
30–31	69	65	88	83	.....do.....	125	94	75.2	31	24.8	
Sept. 3–4	75	65	87	83	.....do.....	250	151	60.4	99	29.6	
6–7	65	58	72	65	.....do.....	87	55	63.2	32	36.8	
10–11	75	65	68	63	.....do.....	116	67	57.8	49	42.2	
13–14	65	60	78	48	.....do.....	42	31	74	11	26	
Total	.....	.....	.....	.....	.....	3,152	[a] 2,200	.....	[b] 952	.....	

[a] 69.8 per cent of total number of moths. [b] 30.2 per cent of total number of moths.

Partial records of temperature and humidity are given in Table III. It is believed from the somewhat scanty data that the night-flying habits of moths are but little influenced by these factors. On the other hand, such meteorological conditions as strong winds, rain, or fog materially restrict flight.

Data as to the total collection of moths, arranged by hours of collection, are set forth in Tables IV and V.

TABLE IV.—*Numbers and percentages of males and of gravid and spent females arranged by periods of collection*

Period ending—	Number of males and females.	Males.		Gravid females.		Spent females.	
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
8 p. m.	23	15	65.2	7	30.4	1	4.4
9 p. m.	354	194	54.8	143	40.4	17	4.8
10 p. m.	372	215	57.8	145	39	12	3.2
11 p. m.	438	331	75.6	88	20	19	4.4
12 p. m.	375	265	70.6	82	22	28	7.4
1 a. m.	391	288	73.6	78	20	25	6.4
2 a. m.	482	365	75.7	75	15.6	42	8.7
3 a. m.	407	307	75.3	63	15.6	37	9.1
4 a. m.	310	220	71	55	17.7	35	11.3
Total	3,150	[a]2,200	.....	[b]736	.....	[c]216	.....

[a] 69.8 per cent of total number of moths.
[b] 23.35 per cent of total number of moths.
[c] 6.85 per cent of total number of moths.

TABLE V.—*Summarized data, arranged by periods of collection*

Period ending—	All moths.				Males.				Females.			
	Number in each period.	Percentage of total number.	Accumulated number at end of each period.	Percentage of total number.	Number in each period.	Percentage of total number.	Accumulated number at end of each period.	Percentage of total number.	Number in each period.	Percentage of total number.	Accumulated number at end of each period.	Percentage of total number.
8 p. m.	23	0.7	23	0.7	15	0.7	15	0.7	8	0.8	8	0.8
9 p. m.	354	11.2	377	11.9	194	8.8	209	9.5	160	17.0	168	17.8
10 p. m.	372	11.8	749	23.7	215	9.8	424	19.3	157	17.0	325	34.8
11 p. m.	438	14.0	1,187	37.7	331	15.0	755	34.3	107	11.2	432	46.0
12 p. m.	375	11.9	1,562	49.6	265	12.0	1,020	46.3	110	11.3	542	57.3
1 a. m.	391	12.4	1,953	62.0	288	13.1	1,308	59.4	103	11.2	645	68.5
2 a. m.	482	15.3	2,435	77.3	365	16.6	1,673	76.0	117	12.3	762	80.8
3 a. m.	407	12.9	2,842	90.2	307	14.0	1,980	90.0	100	10.1	862	90.9
4 a. m.	310	9.8	3,152	100.0	220	10.0	2,200	100.0	90	9.1	952	100.0
Total	3,152	.....	.....	.....	[a]3,200	.....	.....	.....	[b]952	.....	.....	.....

[a] 69.8 per cent of total number of moths.
[b] 30.2 per cent of total number of moths.

It will be seen from these tables that in the first three periods, ending at 10 p. m., there were taken 325 females, or 35 per cent of the total of 952 females and 40 per cent of the total of gravid females. In the same

period, 424 males, or 19 per cent of the total of males, were killed. From 10 p. m. to 4 a. m. the percentage of gravid females declined while that of males and spent females increased. These figures agree with the deductions made by Mr. Geo. G. Ainslie in the excellent paper, "Crambid Moths and Light,"[1] in that a larger percentage of the total number of gravid females and a smaller percentage of the total number of males were captured during the early hours of the night.

That the percentages given here do not approximate more closely those for the material collected at Nashville, Tenn., during the summer of 1915 can be readily understood when it is remembered that Mr. Ainslie's collections were made up practically of one species, *Crambus teterrellus* Zicken, which, as he writes, "is a species without distinct generations and the moths are quite uniformly abundant" during the seasonal period of the adults. The collections made at Hagerstown in the summer of 1918 embraced some sixty-odd species, representing 10 families. Of these 60 species, at least 20 are of economic importance; and several others are likely to prove serious pests if circumstances favor them.

[1] AINSLIE, GEO. G. CRAMBID MOTHS AND LIGHT. *In* Jour. Econ. Ent., v. 10, no. 1, p. 114–123, 3 fig. 1917.

LIFE HISTORY OF EUBIOMYIA CALOSOMAE, A TACHINID PARASITE OF CALOSOMA BEETLES

By C. W. COLLINS, *Entomological Assistant*, and CLIFFORD E. HOOD, *Scientific Assistant, Gipsy Moth and Brown-tail Moth Investigations, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION [1]

In May, 1906, the first adult colony of *Calosoma sycophanta* L. from Europe was liberated in Saugus, Mass. Importations were continued to the close of 1910, and colonizations were made from the material received. During this period rearings were made from the foreign parents, and colonizations were made with the larvæ. During 1912, adults collected in the field for breeding experiments at Melrose Highlands, Mass., died, and from them issued several specimens of a tachinid parasite now known as *Eubiomyia calosomae* Coq. This was the first indication of tachinid parasites from foreign or native material attacking *C. sycophanta* since the introduction of the beetles in 1906, although many beetles arriving dead from Zurich, Switzerland, were placed in tight containers for the purpose of determining whether they were parasitized. No parasites were reared from any foreign material.

The parasite was again recovered from native adults of *Calosoma sycophanta* in 1914. Its importance as a parasite of this economic predacious species was realized, and investigations were begun the following year to secure biological data on the species in order to determine its present and possible future effect on the beetles.

In 1896 Burgess (*2*) [2] collected an adult specimen of *Calosoma calidum* Fab. in Malden, Mass. (although the literature does not give this locality), from which were reared seven flies. These specimens were forwarded to the Bureau of Entomology from Amherst, Mass., and were erroneously labeled and recorded as from that locality. They were determined by Coquillett (*2*) as *Pseudatractocera calosomae* Coq. He stated further that he had bred the same species from *C. peregrinator* Guer. in California. In 1891 Brauer and Bergenstamm (*1*) described a tachinid sent them from the State of Georgia as *Viviania georgiae* B. and B., which they regarded as a distinct species. This form is closely allied to *V. cinerea* Fall., a

[1] The writers are indebted to Dr. L. O. Howard for the loan of specimens from the United States National Museum for comparison; to A. F. Burgess for valuable suggestions in obtaining notes on the life history of the species; to the late Frederick Knab, of the United States National Museum; to C. W. Johnson, of the Boston Society of Natural History, and to R. T. Webber, of the Bureau of Entomology, for comparison and determination of material; and to various men of this bureau stationed at the Gipsy Moth Laboratory, Melrose Highlands, Mass., for collection of beetles yielding parasites.

[2] Reference is made by number (italic) to "Literature cited," p. 497.

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species bred from Carabus in Europe. Coquillett, referring to the same specimens determined as *P. calosomae* in 1896, included them in 1897 (*5, p. 82*) as *Biomyia georgiae* B. and B.

Tothill in 1913 (7) and Burgess and Collins in 1915 (*3, p. 19*) and 1917 (*4, p. 11-12*) recorded rearings of this parasite from *Calosoma sycophanta* under the name of *Biomyia* or *Viviania georgiae* B. and B. A footnote was added by the latter writers to the effect that Dr. C. H. T. Townsend had recently described this species as belonging to a new genus, Eubiomyia, genotype *Pseudatractocera calosomae* Coq. Dr. Townsend's description appeared in 1916 (*8, p. 74*) under this name. Mr. R. T. Webber, of this bureau, has carefully compared specimens bred by the writers at Melrose Highlands, Mass., with the cotype of the above species from the United States National Museum and declares them identical, hence this name is applied to the parasite treated in this paper.

DESCRIPTION OF THE PARASITE

ADULT

The description of *Eubiomyia calosomae* Coq. (Pl. 59, D) as published by Dr. C. H. T. Townsend is included verbatim:

Eubiomyia, new genus.

Genotype, *Pseudatractocera calosomæ* Coqt., 1897, Rev. Tach., 82, resurrected name for *Biomyia* (*Viviania*) *georgiae* Coqt., 1897, l. c., pp., Amherst, Massachusetts (not *Viviania georgiae* B. B.) Holotype, No. 20202 U. S. Nat. Mus., male, reared by A. F. Burgess from adult *Calosoma calidum* at Amherst, Massachusetts.[1]

Differs from *Biomyia* as follows: Female vertex about one-half eye-width. Parafacials, parafrontals, and frontalia all much narrower. Antennæ not so elongate, more narrowed; third joint in both sexes about twice as long as second joint. Frontoorbital bristles closely crowded against frontal bristles. Face more receding, the lower border of head much shorter. Front in both sexes nearly same width. Female without discal macrochætæ on intermediate abdominal segments, male with small ones. Metatarsi of male swollen, especially hind ones, agreeing in this character with *Pseudatractocera*. Apical cell closed, costal spine rather small.

Differs from *Pseudatractocera* as follows: Epistoma shorter and broader. Front less prominent. Parafacials narrower, bare. Hind crossvein nearly in middle between small crossvein and bend of fourth vein. Also in frontal and discal-macrochæta characters given above.

It may be noted here that *Viviania georgiae* B. B. is a *Pseudatractocera*.

The following descriptions of the egg, larva, and pupa of *Eubiomyia calosomae* are by the writers.

EGG

Egg (Pl. 58, A) elongate oval, tapering slightly toward the posterior end. Cylindrical in form from above but flat on lower surface, which is glued to beetle in process of deposition, lower surface being covered with a thin membrane. Color pale yellowish white. Specimens preserved in alcohol and mounted in balsam measured from 0.61 mm. long and 0.34 mm. broad to 0.75 mm. long and 0.4 mm. broad, or an average of 0.68 mm. long and 0.36 mm. broad.

[1] This specimen with others was forwarded to the Bureau of Entomology from Amherst, Mass., but was reared from an adult *Calosoma calidum* collected in Malden, Mass.

The eggs are almost fully developed at the period of deposition, and hatch ordinarily in less than 24 hours. In two instances, one on July 31 and the other on August 1, the eggs were deposited and hatched within 3 hours or less. This is the shortest time recorded. On August 5, 1917, an instance was noted where an egg was deposited, hatched, and the larva had entered its host in less than 13 hours. On August 5 at 9.30 a. m. one egg was observed on a beetle and was removed from its host 24 hours later, still unhatched.

The embryo during hatching makes its way out of the chorion through a small hole (Pl. 58, A) made in the thin membrane on the ventral side. This exit hole is near the anterior end of the egg, the extremity at which the larva emerges from beneath. Fertile eggs in any stage of development if removed from the exoskeleton of the beetles always fail to hatch. This results probably from the immediate drying of the membrane when exposed to the air or the lack of the exoskeleton of the beetle as a base for the embryo to press against in forcing an exit.

LARVA

FIRST INSTAR

One larva less than a day old, dissected from a beetle, measured 2 mm. long and 0.82 mm. broad. Another larva 2 days old measured 2.5 mm. long and 1 mm. broad. Color white. Form elongate, broadest across eighth and ninth segments, tapering gradually towards anterior end. The segments are provided with irregular annular rows of minute black spines which are very indistinct in this stage. Young larva inclosed in a thin, white, membranous encasement or funnel, formed apparently by the growth from the injured or punctured tracheal branch to which it is attached. Funnel soon changes to brown upon exposure to light.

The small larvæ enter their host immediately after hatching, probably through the spiracles. A dissection, August 5 at 2 p. m., of an adult *Calosoma sycophanta* which contained one egg of *Eubiomyia calosomae* deposited in late afternoon or early evening of August 4 revealed a small maggot inclosed in a "funnel" attached to the tracheæ in the region of the metathorax. The anal stigmata of the larva protruded into a hole forced through the wall of the tracheal branch. The spiracles of *C. sycophanta* are composed of an elongate chitinous ring, the inner margin of which is thickly clothed with short hairs directed towards the center. The spiracles measure from 0.8 to 0.9 mm. long and 0.3 to 0.37 mm. broad. In a laboratory experiment, an egg of *E. calosomae* was easily forced into the aperture made by pressing the spiracle open. This leads to the supposition that the young larva enters in this way. Some other laboratory experiments were tried which also tend to strengthen this point. Several partly developed third-instar larvæ were transferred from a parasitized beetle to a normal living specimen. This was accomplished by making an incision in the dorsum of the abdomen of the second beetle and inserting the larva, the wound soon healing. The body of a

normal, well-fed Calosoma is usually quite full of blood, in contrast to the body of a parasitized specimen containing third-instar larvæ. Nevertheless the transferred immature larvæ all died, while the beetles sometimes lived more than a month. These larvæ, though they move about freely in the body cavity of a parasitized beetle where the blood and fats have been greatly reduced, can not withstand the sudden change to full-blooded conditions as found in normal specimens. It is probable that they died from drowning or suffocation.

SECOND OR HIBERNATING INSTAR (PL. 58, D)

Specimens preserved in alcohol ranged from 4.8 mm. long and 1.9 mm. broad to 5.1 mm. long and 2.2 mm. broad, or an average of 4.9 mm. long and 2.1 mm. broad. Color white, form elongate. Larva inclosed in light-brownish tracheal "funnel" which darkens on exposure to light. Funnel of thin membranous tissue darker and thicker at anal end. Base of funnel constricted where it joins walls of trachea and sometimes angled at point of constriction.

The parasite passes the hibernation period as a half-grown or second-instar larva. It remains attached to the tracheal branches of the metathorax in the same position as in the first instar. During July and early August the adults of *Calosoma sycophanta* begin to enter the earth for hibernation, and some of them contain the first-instar larva of the parasite; this larva transforms to the second instar and then ceases its activity and feeding and becomes dormant soon after the beetle has constructed a cavity. Activity of the host and that of its parasite are resumed almost simultaneously in the spring. With parasitized specimens of *C. sycophanta* this activity usually occurs in the latter part of May and the first of June.

THIRD INSTAR (PL. 58, C)

Color grayish white. Varying in size from 8 mm. long and 3 mm. broad to 9.5 mm. long and 3.5 mm. broad, or an average for six alcoholic specimens of 9 mm. long and 3.3 mm. broad. Larva broadest across the eighth or ninth segment, tapering gradually toward anterior and more noticeably toward the posterior end. Each segment except the head is provided with annular spine areas composed of many broken rows of very short black spines, ranging from 6 to 18 rows in each area. These spine areas cover about half the area of the segment, the remaining portion being smooth. Mouth hook (Pl. 58, B) partly visible, especially the two sharp, curved extremities. Anal stigmata rather widely separated in well-fed larvæ. Inner margins almost parallel, rounding at dorsal, ventral, and lateral extremities. Each stigma containing six radial elongate slits. Outer margins of stigmata brownish black and central areas light brown. Stigmata arising out of depression in the anal segment. Larva not found inclosed in tracheal funnel in this stage, as in first and second stages.

When the larvæ have reached the third instar they are found, upon dissection of the host, in all parts of the body cavity from the prothorax to the tip of the abdomen. After the greater portion of blood and fats has been reduced as a result of the constant feeding of several larvæ, the latter move about freely in the body cavity. Their movements increase as the host weakens and approaches death. Feeding and activ-

ity of the larvæ within the body cavity sometimes extend over a period of one or more days after death of the host, which has been hastened by dissection.

The time required for the development of the larva from the hatching of the egg to pupation ranges from 9 to 12 days during midsummer. On June 23, 1917, Mr. F. W. Graham, of this bureau, collected an adult of *Calosoma sycophanta* in the field bearing two eggs of the parasite. By June 24, the chorion of the eggs had disappeared. On July 16 two flies issued. Allowing 11 days for passing the pupal stage, 12 days must have been required to pass the larval instars. In another instance a beetle containing eggs of the parasite was collected in the field, and a dissection of one of the eggs revealed an embryo. The same beetle contained puparia 10 days later. Other breeding records indicate that from 10 to 12 days are passed in the larval stages for the summer generations.

PUPARIUM (PL. 59, A)

Color reddish brown, form elongate, surface smooth when viewed with naked eye but with aid of lens showing 6 to 18 broken annular rows composed of short, thick-set spines to be seen on each segment (Pl. 59, C). These rows appear as continuous, wavy, broken lines, except with high power lens in which case the spines appear distinct but very closely set. Spine area sometimes covering one-half the surface of the segments, but more prominent on anterior and posterior segments. Anal stigmata (Pl. 59, B) protruding above surface of puparium, inner margins almost parallel, and each containing six radiating elongate slits. Puparia ranging in size from 5 mm. long and 2.2 mm. broad to 8.2 mm. long and 4 mm. broad, or an average for eight specimens of 6.6 mm. long and 3.1 mm. broad.

The full-grown larva soon after accomplishing the killing of its host enters the pupal stage. Some of the larvæ in a host containing any number of parasites up to 15 force a hole between the abdominal tergites laterad and pupate just beneath or between the wings and elytra. Of 620 puparia under observation in 1915, 245, or 40 per cent, pupated within the body cavity and 375, or 60 per cent, between the dorsum of the abdomen and the elytra. The maggot pupates very shortly after the death of its host, the period being hastened somewhat where dissections are made which expose the tissue of the host and the maggot to the drying of the air. When the host is opened and contains maggots practically full grown, pupation usually follows in from 10 to 36 hours.

The time passed in the pupal stage ranges from 9 to 15 days. An average of 12 days in midsummer was required in 1915 by many specimens under observation; 11½ days were required in 1916 and 11 days in 1917.

DISTRIBUTION OF THE PARASITE

Eubiomyia calosomae has been bred from collections of *Calosoma sycophanta* from many towns in eastern Massachusetts and from one or more towns in Maine, New Hampshire, and Rhode Island. Its known

distribution occurs as far north as York County, Me.; Merrimack County, N. H.; as far west as Worcester County, Mass.; and as far south as Plymouth County, Mass., and Providence County, R. I. It undoubtedly occurs in New England outside these limits, for the distribution of *C. sycophanta* extends beyond the above-named localities and into Connecticut. It has been bred also from *C. frigidum* Kirby and *C. calidum* Fab., which were collected within the above-named limits. Since the two latter species occur as far south as Georgia and Texas and as far west and north as Nebraska, North Dakota, and Canada, the parasite is likely to be found in any part of this extensive territory.

It was first thought when this species was bred from *Calosoma sycophanta* in 1912 and 1914 that it might be *Viviania cinerea* Fall. of Europe, introduced from that continent with the beetles during the years 1905 to 1910. The first shipment of beetles was received from the late Dr. G. Leonardi, of Portici, Italy. Practically all later shipments were sent by Miss Marie Ruhl, Zurich, Switzerland. These were shipped in tight boxes, and when these were opened on this side the specimens and packing material were examined for parasites. In most instances the dead adults were inclosed in a jar containing earth in order to rear any possible parasites. The table shows that 4,046 living specimens and 2,097 dead specimens of *C. sycophanta* were received from Europe and no signs of parasites were observed. It might be added that 348 adults received between 1905 and 1910, and pinned at that time, were later dissected with negative results.

The data at hand tend to point to the fact that the species was not introduced from Europe but is native to New England, being first bred by A. F. Burgess in 1897 from *Calosoma calidum* and later found more abundantly in *C. sycophanta*.

TABLE I.—*Number of living and dead specimens of Calosoma sycophanta received from Europe*

Year.	Living.	Dead.
1905	1	215
1906	693	128
1907	967	1,125
1908	675	113
1909	405	39
1910	1,305	477
Total	4,046	2,097

EQUIPMENT USED IN BREEDING EXPERIMENTS

During 1915, when the first extensive breeding experiments were begun, the flies issuing from the parasitized beetles were inclosed in small wooden, glass-covered trays 12 by 12 by 3½ inches with a bottom

upon which earth was placed. Two or more specimens of Calosoma were inclosed with some caterpillars for food. The flies were offered sugar and water absorbed in a sponge and also sprayed on foliage. About 372 flies were used in the experiments, but no copulation or oviposition was observed that year.

In 1916, although fewer flies were reared than in 1915, a show case was modified so as to answer for a breeding cage. It measured 31½ by 21½ inches and was 11 inches deep. The glass was removed from the ends and fly screen substituted. The top and one side were of glass, and the other side contained two sliding doors. Shallow pans of earth were inclosed for the beetles, and flowers and sweetened water for the flies. More freedom was given the host and its parasites, and better results attended these efforts. Fertile eggs were secured in 1916, 1917, and 1918.

The beetles parasitized in the breeding cage were transferred to large glass battery jars partly filled with loam for hibernation and also to box cages set in the ground. These cages were provided with a top and bottom of copper wire of fine mesh.

PARASITES BRED FROM FIELD COLLECTIONS OF CALOSOMA SYCOPHANTA

During the seasons of 1915, 1916, 1917, and 1918 large field collections of beetles were made in eastern Massachusetts where the species had become abundant. These collections were made for the dual purpose of securing specimens for colonization in new and outside territory infested with *Porthetria dispar* and of studying the parasite attacking them. During 1915, 15,322 beetles were collected, and 204, or 1.3 per cent, died of parasitism. At that time such large numbers were collected for colonization that it was impracticable to hold all of them in cages until all died that would have succumbed to parasitism, hence the percentage is probably slightly lower than it normally should be. During 1916, 465 beetles were collected. These were fed in cages long enough for all those affected with parasites to have died. Sixteen beetles, or 3.4 per cent, died from parasitism. During 1917, 4,679 beetles were collected and confined in large cages. Sixty-three of these, or 1.3 per cent, died from parasitism. Six hundred of this lot were forwarded to Mr. L. S. McLaine, of the Department of Agriculture, Dominion of Canada, located at Frederickton, New Brunswick, for colonization. Mr. McLaine, upon request, held and fed the beetles in cages until July 21. During this period 20 died, and 18 flies issued from the 20 dead. This would indicate that from 6 to 10 died of parasitism, since an average of about 3 flies usually issue from each beetle. A total of 6,072 beetles were collected in 1918, and only 16, or 0.26 per cent, died from parasitism.

During these years the dead beetles collected in the field or dying in the cages at the laboratory were dissected or isolated in vials or jars for issuance of the parasites. The data collected in 1915 showed that the

204 parasitized beetles collected contained 763 puparia, from which 641 flies issued, 122 dying in the pupal stage. The highest number of puparia contained in one beetle was 16 and the lowest 1. This gives an average of 3.7 puparia in each parasitized beetle, from which an average of 3 flies issued. The data secured in 1916 showed an average of 3 puparia per beetle, from which an average of 2.8 flies issued.

All adults of *Calosoma sycophanta* collected in the field for colonization purposes in 1917 and 1918 were examined individually for eggs of the parasite as soon as they arrived at the laboratory. Although the eggs hatch in a short time, the chorion sometimes remains glued to the beetles for a few days. A total of 5,707 adults were collected in 1917 between June 20 and July 24. Five beetles were found containing eggs of the parasite on the following dates: June 23, June 25, and July 6. During 1918, 6,072 adults were collected in the field between May 28 and July 19. Two beetles containing eggs were collected on June 5 and July 3. Those eggs found on June 5 apparently were deposited by flies of the winter generation and those of July 3 by flies of the summer generation.

PARASITES BRED FROM FIELD COLLECTIONS OF CALOSOMA CALIDUM FAB., CALOSOMA FRIGIDUM KIRBY, AND CARABUS NEMORALIS MÜLL., 1912–1918

Small collections of adults of *Calosoma calidum* Fab., *Calosoma frigidum* Kirby, and *Carabus nemoralis* Müll. were made almost every year in eastern Massachusetts and southern New Hampshire between 1912 and 1918 and confined in jars of earth at the laboratory. From them specimens of *Eubiomyia calosomae* were bred occasionally. Since some of the early rearings from these collections were more or less accidental, it is thought advisable to give the percentage of parasitism as based on the total collections of each species for the 7-year period. During this period 92 specimens of *Calosoma calidum* were collected, 3 specimens, or 3.3 per cent, of which were parasitized. A total of 238 adults of *Calosoma frigidum* were collected, of which but 1, or 0.42 per cent, yielded parasites; and 169 adults of *Carabus nemoralis* were collected, 1 of which, or 0.59 per cent, was parasitized. It might be added that in 1912 when 49 adults of *Calosoma calidum* were collected, 3, or 6 per cent, yielded to parasitism and in 1915, when only 3 adults of the same species were collected, 1, or 33 per cent, later died of parasites.

These records tend to indicate that the parasite is slightly more effective on *Calosoma calidum* than on *C. sycophanta*. This may explain in part why the former species is less common in New England than formerly.

LIFE-HISTORY STUDIES

OVIPOSITION

The eggs are deposited by the female on the exterior surface of Calosoma adults, being glued to several parts of the exoskeleton. They are deposited singly and in patches of 2, 3, and 4. Eggs have been found attached to

elytra, dorsum and lateral portions of prothorax, ventral portion of abdomen, and femora of *Calosoma sycophanta*.

During the season of 1916, summer generation flies in cages deposited 95 eggs on 38 beetles, and in 1917, they deposited 177 eggs on 68 beetles, or an average of more than 2 per beetle for each season. Of those deposited in 1917, 61 eggs were found on the abdomens, 52 on the prothoraces, 20 on the metathoraces, and scattering ones on the legs and elytra of the various beetles. The largest numbers deposited on one beetle in the breeding experiments were 10 and 9 during 1916 and 1917, respectively.

The period of oviposition in the cage experiments was from July 28 to August 16 in 1916, from July 22 to August 12 in 1917, and from July 19 to July 27 in 1918.

The flies in the breeding cages are most active during the late afternoon and early morning—the times at which adults of Calosoma are least active. Oviposition was observed in a cage on August 6, 1915, at 5.50 a. m. and at 6.35 a. m. The flies were observed to run after the beetles as early as 4 a. m.—the breaking of day. Eggs have also been found deposited on beetles between 5 p. m. and dusk. The female fly lights near the beetle and mounts upon the dorsum of its host either by crawling upon it or by a short flight. The beetles in cages sometimes show little disturbance from the presence of the parasites near or upon them. Occasionally they are seen to run slowly away from the active flies.

COPULATION

Attempts were made to observe copulation of this species at various periods during the season in 1915 and 1916. Pairs of flies were inclosed in lamp chimneys and glass jars and provided with foliage and a sugar solution. Observations were made frequently, the flies being separated at night and returned to the receptacle each day, but negative results attended these efforts until 1917.

On July 21, 1917, one male and three female flies issued in a vial. The male, which was less than 24 hours old, was transferred to a vial containing another female of the same age. Copulation ensued within two minutes, continuing for a period of six minutes, whereupon the pair was transferred to the large breeding cage with other specimens. After this period many fertile eggs were secured during the progress of the experiment.

LONGEVITY

On July 10, 1915, two males and two females, newly issued specimens, were inclosed in a wooden tray with glass cover to obtain data on the longevity of the species. Foliage and cotton saturated with sugar solution were also supplied in the tray. The length of life was as follows: One

male lived 4 days, one male 5 days, one female 9 days, and one female 16 days.

Another similar experiment was started on July 14, 1915, with eight flies inclosed in a lamp chimney. The length of life was as follows: One fly lived 7 days, one fly 8 days, two flies 11 days, one fly 23 days, one fly 24 days, one fly 26 days, and one fly 30 days. The minimum in this experiment was 7 days and the maximum 30 days, or an average of 18.

HIBERNATION

Eubiomyia calosomae hibernates in the second larval instar (Pl. 58, D) within the body cavity of Calosoma adults. The small maggot, after hatching, enters the body probably through the spiracles, for the first-instar larva is found attached to the tracheal branches in the metathoracic region. It feeds in this location for a few days and enters the second instar about the time the beetles enter the earth for hibernation.

Four eggs were deposited upon an adult in the breeding cage on July 26, 1917, and the beetle was immediately transferred to a jar of earth into which it descended for hibernation about August 1. The beetle was dissected February 9, 1918, and was found to contain four second-instar larvæ securely attached posteriorly to the tracheal system of the beetle and inclosed in a tracheal funnel. The location of these larvæ was within the body cavity above the metasternum and lying longitudinally with the anal stigmata pointing toward the anterior end of the beetle.

When spring arrives the parasitized beetles, pressed by weakness from loss of blood and fats, begin early in the season to wend their way upward to the surface of the ground in search of food. The parasites are probably feeding all the while during this activity, further weakening their host; and death results in the course of two or three days. The dead parasitized beetles are usually found on the surface of the earth. It is quite probable that they sometimes die before wholly emerging, but it has been proved by laboratory experiments that flies issuing under such conditions are capable of forcing their way through a few inches of earth without harm to themselves.

The parasitized beetles emerged on May 25, 1916, and about June 1, 1917; but the maximum normal field emergence of specimens of *Calosoma sycophanta* was about June 10 to 15 during those years.

NUMBER OF GENERATIONS AND PERIOD OF EMERGENCE OF FLIES

Eubiomyia calosomae passes through two complete generations a year on *Calosoma sycophanta* as a host and in some instances, where beetles remain active for a period of one week or more after becoming parasitized, through a partial third generation. On May 27, 1915, H. W. Allen collected a female of *Eubiomyia calosomae* at Lunenburg, Mass. This is the earliest known seasonal record of issuance of adults in the

field. From adults of *C. sycophanta* collected in the field in late July, 1915, and allowed to hibernate in large cages the following winter, one parasitized female emerged on May 25. On May 26 it was dead, and when dissected contained a puparium. The fly issued June 6, 1916. Between August 4 and 10, 1916, eggs were deposited upon two beetles and the latter allowed to hibernate in ground cages. On June 5, 1917, the two beetles were found dead on the surface of the earth from which they had recently emerged, Each contained one puparium. One fly issued from these on June 9, 1917. The flies mentioned above were of the winter generation, or parent stock of the summer generation or generations. The first issuance of the summer generation from parasitized adults of *C. sycophanta* from field collections was on July 6, 1915, July 10, 1916, and July 11, 1917. The corresponding last dates of issuance for these years were August 21 for 1915, July 28 for 1916, August 3 for 1917, and as late as August 21 in a reproduction experiment in 1917. These dates differed both because of variation in the number of parasitized beetles collected each year and because of variation in the seasons.

Three female specimens of *Calosoma sycophanta* were parasitized in a breeding cage late on July 26 or early on July 27, 1917, and were transferred to jars of earth for hibernation. The first female soon entered the earth, making its cavity 5 inches below the surface. The other two females remained active on the surface of earth in the jar and fed for about a week. On August 4 the first beetle was removed from its cavity, killed, dissected, and found to contain two second-stage or dormant hibernating maggots. The second and third females were found dead on the surface of the earth in the jar on August 4 and 7, respectively. These latter beetles contained active third-stage maggots from which eight flies issued between August 15 and 21. Similar records were secured in 1918. Most of the flies issuing in the field after July 20 to 25 probably perish for lack of a suitable host in a species of Calosoma, since practically all the adults of the latter have entered the earth for hibernation by this time. Presumably the first issuing flies of the summer generation oviposit upon beetles, from which, if they remain active for a sufficient period, there later issue flies of the second summer generation.

Table II shows the daily issuance of flies for three seasons. It will be observed that the maximum issuance of flies during average seasons occurred between July 6 and August 1. Adults of *Calosoma sycophanta* are most abundant in the field from June 15 to July 10, decreasing in abundance very rapidly after the latter date. Active beetles are very rarely met in the field after August 1, hence the late issuing flies find a great scarcity of hosts upon which to oviposit. This in turn accounts for the low percentage of parasitism generally found among the beetles emerging from hibernation in late May and June.

TABLE II.—*Number of parasites issuing and dates of emergence from adults of Calosoma sycophanta, 1915, 1916, and 1917*

Date.	Number of parasites issued daily.			Generation.
	1915.	1916.	1917.	
June 6	.....	1	.....	Winter.
9	.....	.....	1	Do.
July 6	6	.....	.....	Summer.
7	5	.....	.....	Do.
8	19	.....	.....	Do.
9	10	.....	.....	Do.
10	9	2	.....	Do.
11	20	1	4	Do.
12	25	4	.....	Do.
13	20	.....	3	Do.
14	43	3	5	Do.
15	48	.....	.....	Do.
16	35	3	11	Do.
17	27	6	6	Do.
18	21	2	6	Do.
19	39	4	11	Do.
20	12	3	11	Do.
21	2	1	7	Do.
22	13	3	9	Do.
23	12	2	8	Do.
24	1	2	1	Do.
25	5	4	5	Do.
26	4	3	11	Do.
27	14	2	9	Do.
28	18	6	.....	Do.
29	7	.....	11	Do.
30	9	.....	1	Do.
31	7	.....	.....	Do.
Aug. 1	4	.....	.....	Do.
2	1	.....	2	Do.
3	2	.....	1	Do.
4	1	.....	.....	Do.
6	3	.....	.....	Do.
7	3	.....	.....	Do.
9	5	.....	.....	Do.
13	1	.....	.....	Do.
16	8	.....	.....	Do.
17	1	.....	.....	Do.
18	1	.....	.....	Do.
19	9	.....	.....	Do.
21	4	.....	.....	Do.
Total	474	52	123	

The late issuance of flies as recorded in 1915 was undoubtedly the result of the issuance of a partial second generation at a time when it was improbable that suitable hosts could be found active in the field. According to the record secured in the breeding experiment in 1918, and cited above, it would seem that the activity of the progeny of the summer generation of parasites is entirely dependent upon the activity of the host. That is, if beetles parasitized by flies of the summer generation immediately become inactive and seek dormancy, the larvæ of the

parasite apparently do likewise. But, on the contrary, if the beetles continue active and feed for a period of 10 days to 2 weeks after becoming parasitized, the larvæ continue to develop until they reach the adult stage, there being thus a partial second summer generation.

HOSTS

Parasites have been bred from adults of *Calosoma sycophanta*, *C. frigidum*, and *C. calidum* collected in the field in New England. Large numbers were bred from the first species because of its great abundance and fewer from the other two species because of their rarity in comparison with *C. sycophanta*. Eggs have also been deposited on *C. wilcoxi* in the breeding cage and the parasite reared to the second or hibernating larval instar. The host which died in hibernation contained two larvæ.

One specimen of *Carabus nemoralis*, collected by P. S. Coffin, Framingham, Mass., May 18, 1916, was dissected and found to contain a large puparium of *Eubiomyia calosomae*. On July 26, 1918, two eggs were deposited by the parasites in a breeding experiment upon a specimen of *C. nemoralis*. This parasitized host later entered the earth for hibernation.

It is very probable that this parasite attacks some species of Calosoma and Carabus occurring in New England, other than the ones here recorded, but the remaining species of these genera are comparatively rare. It is also possible that *Eubiomyia calosomae* may be responsible in a measure for the scarcity of some of the remaining species of the genera.

Larvæ of *Calosoma sycophanta*, *C. frigidum*, and *C. scrutator* were exposed to the flies in the breeding experiments but without results. The flies paid no attention to the larvæ and made no attempt to oviposit upon them. A collection of 1,546 large larvæ of *C. sycophanta* was made during the summer of 1915 and reared to maturity to determine whether *Eubiomyia calosomae* attacks the larvæ, but no parasites were reared. *Doryphora decemlineata*, *Lachnosterna* sp., *Porthetria dispar*, *Euvanessa antiopa*, *Hyphantria cunea*, and *Estigmene acraea* larvæ were also exposed in the breeding cages but without action on the part of the flies.

NATURAL ENEMIES

On November 6, 1915, one dead adult of *Calosoma sycophanta*, collected in Randolph, Mass., contained one puparium of *Eubiomyia calosomae* Coq. On December 14, the puparium was dissected and found to contain a large larva of *Chalcis* sp. as determined by Mr. C. F. W. Muesebeck, formerly of this bureau. Other puparia have been dissected with no further indications of secondary parasitism.

ECONOMIC STATUS OF THE PARASITE

This parasite has two complete generations and a partial third per year, adults issuing in May and June and again in July and August. After a few days the flies which issue early have an abundance of hosts

upon which to oviposit because of the emergence of *Calosoma sycophanta* from hibernation. The summer generations issuing in July and August are more abundant than the first generation, but they find comparatively few beetles to parasitize because the beetles begin to enter hibernation about July 15 and decrease very rapidly in numbers above ground after that date. There is an abundance of flies issuing after this period that must perish for want of a host unless they attack some host other than species of Calosoma or Carabus. This probably accounts for the present limits to the increase of the parasite.

Additional recorded hosts for this parasite are comparatively rare in the New England section where *Calosoma sycophanta* abounds. It is difficult to predict the future effect of the species on *C. sycophanta* since future rarity or abundance of other related hosts may have a great bearing on the subject; but it is believed that the parasite will not become much more abundant than it is at present. The average of 3.3 per cent parasitism secured from the small collections of *C. calidum* indicates that the parasite is more effective on *C. calidum* than on *C. sycophanta*, upon which the highest parasitism was 3.4 per cent, secured in 1916, or an average of 1.12 per cent for a period of several years. Should *C. calidum* by chance become very abundant in eastern Massachusetts and southern New Hampshire where *C. sycophanta* is now very abundant, the depredations of the parasite might also increase, since a longer breeding season for the latter would be furnished. *C. calidum* emerges from hibernation in the territory mentioned about May 1 to 15, and *C. sycophanta* about June 1 to 15. It is therefore apparent that the two species of hosts which occur abundantly in the same locality might increase the abundance of the parasite materially.

SUMMARY

Eubiomyia calosomae has two full generations per year and a partial third under favorable seasonal conditions. The eggs hatch in from 3 to 24 hours, the larvæ develop in from 9 to 12 days, and the pupæ in from 9 to 18 days, making it possible for a generation to develop fully in from 20 to 25 days.

The first record of the rearing of this parasite in this country was from a specimen of *Calosoma calidum* in 1896. If one judges from the literature on the species and the small representation of specimens in the collections which were taken with the net, one would infer that the species is rare in the East; but this may be due to the peculiar habits of the adults in the field, which are not well known. The species seems to have attracted little attention between 1896 and 1912, when it was first bred from *C. sycophanta*, six years after the successful introduction of the latter species into New England. During the seasons from 1915 to 1918, over which period a study was made of its life history, the highest parasitism upon *C. sycophanta* was 3.4 per cent in 1916,

and an average of 4.4 per cent upon *C. calidum* for a period of years. The abundance of the parasite of the summer generations occurs at a time when adults of *C. sycophanta* and other species of Calosoma are beginning to enter the earth for a period of dormancy followed by hibernation. This doubtless tends to limit the opportunity for increase under present conditions. The data thus far accumulated show that the parasite has not yet caused a serious handicap to the abundance and usefulness of *C. sycophanta* in New England.

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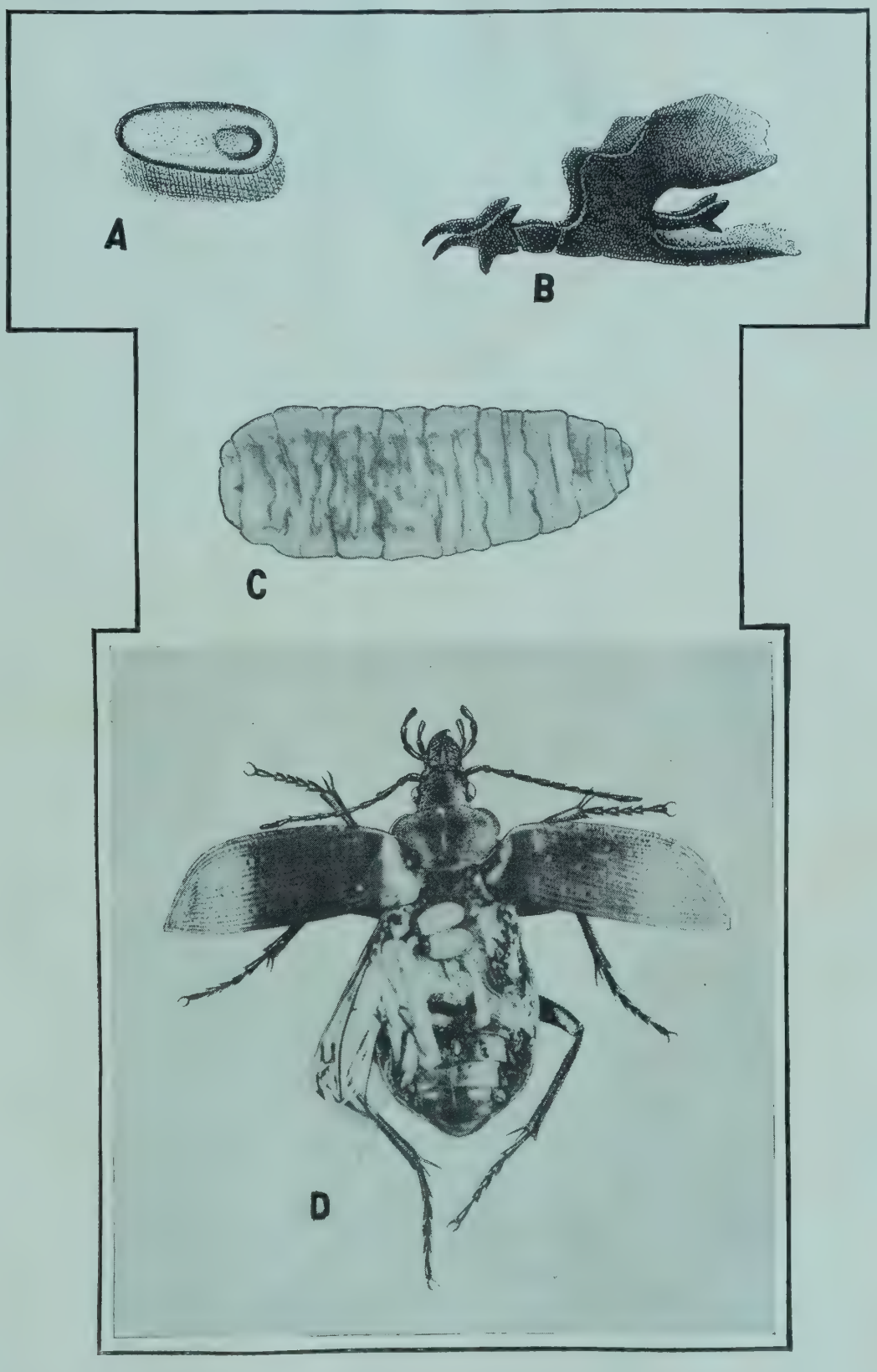
PLATE 58

Eubiomyia calosomae:

A.—Egg, showing flat side and exit hole of larva. Much enlarged.
B.—Mouth hook of third-stage larva. Much enlarged.
C.—Third-stage larva after preservation in alcohol. Enlarged.
D.—Adult of *Calosoma sycophanta* containing two second-stage or hibernating larvæ of Eubiomyia. × 1⅔.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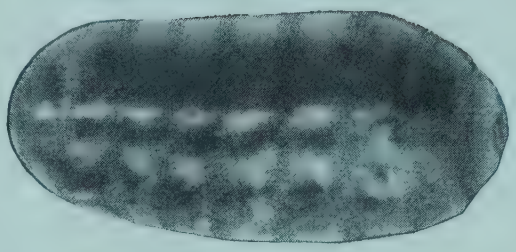

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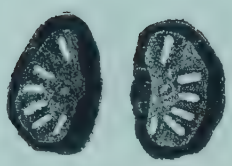

B

C

D

PLATE 59

Eubiomyia calosomae:

A.—Puparium. × 7.
B.—Anal stigmata of puparium from slide mount. Much enlarged.
C.—Section of spine area on segment of puparium. Much enlarged.
D.—Adult female. × 7.

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DETERMINATION OF NORMAL TEMPERATURES BY MEANS OF THE EQUATION OF THE SEASONAL TEMPERATURE VARIATION AND A MODIFIED THERMOGRAPH RECORD

By F. L. WEST, *Physicist*, and N. E. EDLEFSEN and S. EWING,[1] *Assistant Physicists, Utah Agricultural Experiment Station*

In the fall of 1918 Logan, Utah, was paving some of its streets. Because of the labor shortage, the work had been delayed and it was feared that freezing weather would set in before the work could be completed. The city engineer asked the writers what temperature Logan would be likely to experience during the third week of November between 4 and 6 p. m. They could not tell him.

In attempting to answer this question the writers were attracted to a study of seasonal temperature change and also to the change in temperature as the day advances. These temperature changes occur rather gradually and regularly. It becomes slowly warmer as the day advances; and after the setting of the sun the air cools, until just before sunrise, when it begins to warm again. This is repeated each day. The mean daily temperature gradually rises as the summer approaches, reaches a maximum, begins to fall, and reaches a minimum in the winter. The writers undertook to determine, from the temperature observations of the Weather Bureau, the law connecting the temperature and the time, for the purpose of determining the probable temperature at a particular place for a particular day and a certain hour of that day.

LITERATURE

Lambert, Weilenmann, Maurer, Lamont, Trabert, and Angot worked on the problem of the diurnal change in temperature in an attempt to give a mathematical expression for the temperature change in which the constants of the equation had a physical significance.[2] The upper part,

[1] The authors wish to thank Dr. Willard Gardner, Associate Physicist at the Utah Agricultural College, for helpful suggestions on this problem.

[2] PERNTER, J. M. PRESENT STATUS OF OUR KNOWLEDGE OF THE CAUSES OF THE DIURNAL CHANGES IN TEMPERATURE, PRESSURE, AND WIND. *In* Mo. Weather Rev., v. 42, no. 12, p. 655–665. 1914. Notes and references, p. 662–665.

Discusses in detail articles by Lambert, Weilenmann, Maurer, Lamont, Trabert, and Angot. The original of these articles not available to the author of this paper.

or daytime period, has so many physical factors involved that the problem was very difficult. The night interval, or lower part of the curve, they found to be logarithmic, the constants of the equation having a physical significance. The equation of the upper part of the curve, the equation of the lower part of the curve, and a combined equation of the two—partly empirical—have been worked out. Occasionally a section of the country experiences a warm winter or a cold summer.[1] These temperature courses have been studied by Gawthrop.[2]

ANNUAL TEMPERATURE CHANGE

The seasonal change in temperature is due to the fact that the earth is moving around the sun in an elliptical orbit with a variable speed, its axis of rotation making an angle of 23½° with the plane in which it revolves. The 24-hour temperature variation is due to the rotation of the earth on its axis.

If the rate of emission of heat by the sun were to remain constant and if the rate of rotation and of translation of the earth be the same each year—the path of the earth around the sun being the same each year—and if the diathermancy of the atmosphere were to remain constant with no evaporation or condensation, the sequence of temperatures of one year would be very nearly exactly that of any other year, and the thermograph record, for April 1, for example, would be the same every year. On cloudless days this is nearly realized. The foregoing conditions are nearly realized except for the diathermancy of the atmosphere, which varies because of evaporation and cloud formation. It is because of the passage of cyclones and anticyclones—storm and fair weather areas—across a section with the accompanying rain or snow, the dissolving of clouds, or evaporation of rain, that we have temperature departures from normal.

In order to determine the normal change in temperature with the time over the entire year, it would be necessary to get the mean temperature for every hour of the year for enough years to eliminate the irregularities due to storms, and then make a plot of these 8,760 (24 × 365) hourly temperatures and determine the equation of the curve. This is possible, but it is obviously a very tedious operation. The same result may be obtained much more easily by dividing the problem and considering the seasonal temperature change and the daily change separately. In other words, it will be shown how to determine the mean daily temperature, and this value will be multiplied by a certain percentage in order to get the value for a certain hour of the day.

However, the arid West, comprising the section between the Rocky and Sierra Nevada Mountains, has only about 10 inches of rainfall and little cloudiness, a humidity of but 50 per cent, and 300 days of the year

[1] PERNTER, J. M. OP. CIT.

[2] GAWTHROP, Henry. TEMPERATURE COURSES. *In* Mo. Weather Rev., v. 35, no. 12, p. 576–578, 1 fig. 1907.

without rain. In addition, fully one-fourth of the land area of the earth is equally dry. The departures from the normal temperature, therefore, are least for these areas; and the method to be developed in this paper is most useful for them in predicting actual temperatures.

Figure 1 represents the mean monthly temperatures for several widely separated cities of the United States and shows how the temperature changes as the seasons advance. The curves are somewhat alike. For Seattle and San Francisco they are flatter than for the others, showing that the difference in temperature between summer and winter is slight. These cities are said to have an equable or oceanic climate because of their relative position to the ocean with its high heat capacity and the prevailing westerly winds. It is probable that for cities with the same mean annual temperature and with the same difference in temperature

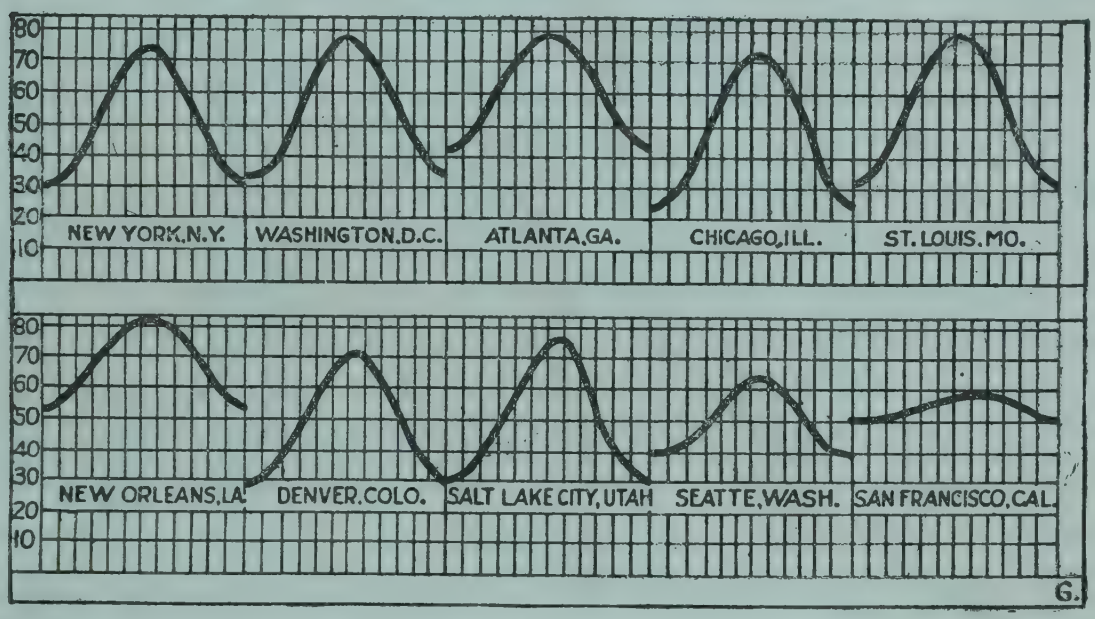

FIG. 1.—Synoptic chart of annual temperature marches at selected stations in the United States.

between summer and winter the curves would be just alike. For cities with the same annual variation the curves would have the same slope.

Figure 2 shows the change in temperature with the season in Utah and represents the mean monthly temperatures for the State.

Any single-valued periodic function can be expressed by an infinite trigonometric series, or Fourier series, of the form

$$T = a + b \sin x + c \sin 2x + d \sin 3x + \ldots\ldots$$
$$+ e \cos x + f \cos 2x + g \cos 3x + \ldots\ldots\ldots \quad (1);$$

or if it is desirable to express it in terms of the cosine only, it takes the following form:

$$T = a + b \cos(\theta - c) + d \cos 2(\theta - e) + f \cos 3(\theta - g) + \ldots \quad (2),$$

the contents a, b, c, d, etc., determining the shape of the particular curve. The method is well known to mathematicians and is explained in the larger texts on calculus under the head of Fourier's series.

The following is the equation of the curve of figure 2, which represents the seasonal temperature change for Utah:

$$T=48.5-20.91\cos\theta-1.28\cos 2\theta-0.67\cos 3\theta+\ldots\ldots$$
$$-7.57\sin\theta+2.38\sin 2\theta-0.83\sin 3\theta+\ldots\ldots\ldots\ldots \quad (3),$$

or in terms of one trigonometric function only:

$$T=48.5-22.2\cos(\theta-19^\circ-54')-2.70\cos 2(\theta-149^\circ-5')$$
$$-1.03\cos 3(\theta-17^\circ-3')+\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4),$$

where T represents the temperature at the time θ. The first constant in each of these equations (48.5) is the mean annual temperature for the State, expressed in degrees Fahrenheit.

In the last two columns of Table I it is shown that for the 30 county seats of Utah the mean annual temperatures differ from the mean for

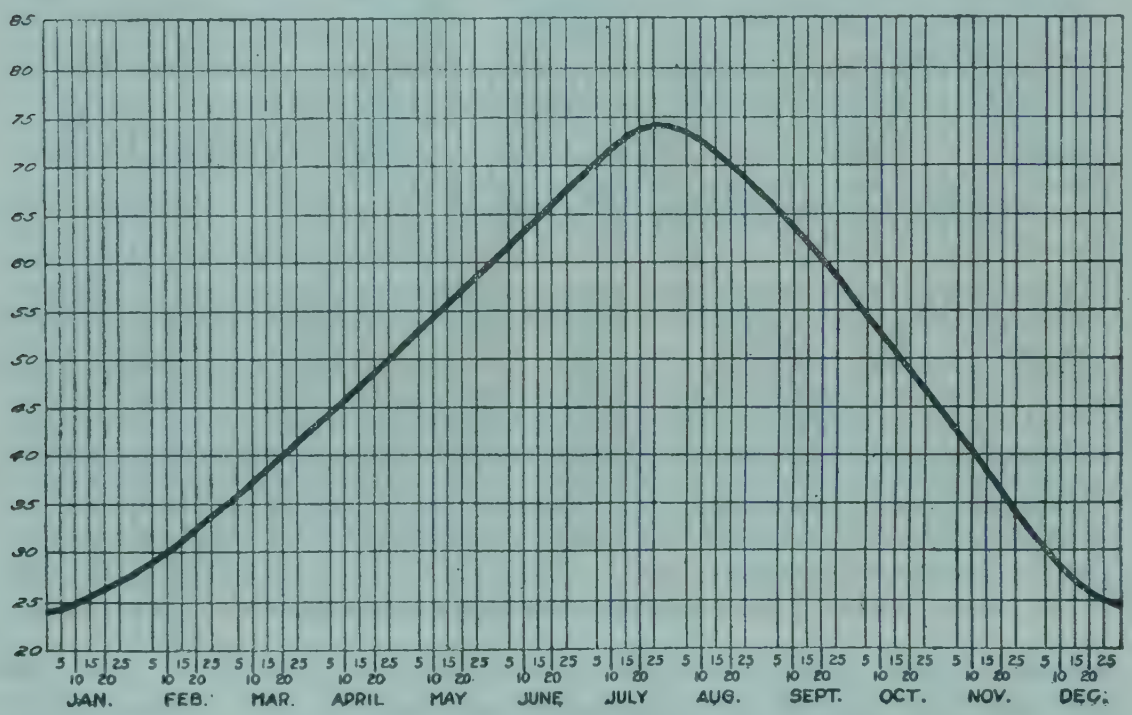

FIG. 2.—Mean monthly temperatures for Utah.

the State by from 0° to 10° F., with a mean departure of 4°, and that the mean annual range for these towns differs from the State range 0° to 9°, with a mean departure of 4°. The first constant in the equation simply moves the graph as a whole up or down the page without changing its shape. Inasmuch as the annual range in temperature—the difference in temperature between summer and winter—is nearly the same for all these county seats, making all their curves of approximately the same shape, the above equation can be used for any of Utah's towns or any other place that has the same annual range by replacing the first term by the mean annual temperature for the place in question.

To determine the mean daily temperature on April 1 for Salt Lake City, change the date to degrees by dividing the number of days that have elapsed since January 1 by 365 and multiply by 360, which gives

90, and use 90 for θ in the equation, and for the first term of the equation use the mean annual temperature for Salt Lake City (52° F.) instead of 48.5°, and by means of a table of cosines calculate the desired temperature from formula 4.

TABLE I.—*Mean monthly temperatures with daily and annual ranges (in degrees Fahrenheit)*

Station.	County.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Annual.	Mean daily range.	Mean annual range.
Beaver	Beaver	29	30	39	54	61	61	68	67	58	48	39	27	48	31	39
Castle Dale	Emery	19	26	38	46	54	63	69	68	59	47	37	23	46	34	50
Corrinne	Box Elder	24	30	40	50	59	70	78	74	65	51	37	28	51	29	54
Duchesne	Duchesne	16	22	35	46	52	61	68	67	58	46	33	19	44	30	52
Escalante	Garfield	26	33	41	48	55	65	69	68	60	50	40	29	49	31	43
Farmington	Davis	29	33	41	49	56	65	73	71	60	50	40	31	50	25	43
Fillmore	Millard	31	34	42	50	58	68	75	74	65	53	42	30	52	33	45
Ft. Duchesne	Uinta	14	18	34	46	54	62	68	67	58	45	33	16	43	33	55
Heber	Wasatch	21	24	34	44	52	59	66	66	55	45	35	22	44	33	46
Henefer	Summit	22	26	35	44	51	58	65	64	55	45	35	24	44	34	43
La Sal	San Juan	25	28	35	45	51	61	67	66	57	46	37	25	45	24	42
Levan	Juab	25	29	37	46	53	64	71	70	60	48	37	26	47	27	47
Loa	Wayne	25	24	32	41	49	59	66	63	53	41	31	21	42	35	41
Logan	Cache	24	27	36	48	54	63	72	71	61	50	38	26	47	22	47
Manti	Sanpete	25	28	38	46	55	63	69	68	59	48	37	25	47	30	45
Marysvale	Piute	28	31	38	45	51	60	66	65	58	47	37	25	46	34	41
Meadowville	Rich	22	22	29	41	49	57	65	63	54	44	33	24	42	....	43
Moab	Grand	29	36	43	55	64	72	78	75	66	54	41	30	53	31	50
Morgan	Morgan	23	27	36	46	52	59	66	66	56	46	36	23	45	31	43
Ogden	Weber	29	32	41	51	59	69	77	75	63	51	40	31	52	....	47
Parowan	Iron	29	32	39	47	55	64	71	67	60	49	41	29	49	30	41
Provo	Utah	27	31	40	48	57	64	72	70	60	49	39	26	49	31	46
Richfield	Sevier	27	32	40	48	55	63	70	68	59	48	38	28	48	35	41
Salt Lake City	Salt Lake	29	33	42	50	57	67	76	75	64	52	41	32	52	20	47
St. George	Washington	38	42	49	57	66	76	82	81	71	59	46	38	59	37	55
Tooele	Tooele	29	33	40	49	55	65	73	73	63	50	40	31	50	22	44
Woodruff	Rich	15	17	28	40	47	55	61	60	51	41	30	19	39	33	45

Most of the interior cities of the United States have an annual variation in temperature differing from that of Utah by not more than 10° F. (see fig. 1). Since it is this factor that largely determines the shape of the curve, approximate mean daily temperatures can be obtained for any of these cities by means of equation 4 by using the mean annual temperature for the particular city in place of the first term (48.5).

The series converges rather rapidly, the omission of the fourth term making an error of one-half of 1 per cent and the omission of the third and fourth terms making an error of 2 per cent.

DAILY TEMPERATURE CHANGE

The difference in temperature between day and night in Utah—that is, between the maximum and the minimum for the 24 hours—is about 30° F. in summer and only about 15° in winter. This is because the sun attains a greater altitude at noon in the summer and heat is being received from the sun more rapidly on unit area than in the winter time, which causes the temperature to rise faster and reach a higher value for the same time period. Cooling takes place faster at night in the

summer time than in winter because of the greater difference in temperature between earth and upper atmosphere in the summer. The daily temperature change curve, or thermograph record, is very much flatter in winter than in summer. Figure 3 clearly shows this fact. Because this curve for the same place is continually changing as the season progresses, no direct attempt was made to determine its equation.

Figure 4 shows thermograph records from four widely separated cities of the United States. It is to be observed that the hottest time of day is about 4 o'clock—it varies with the season—instead of noon, and the

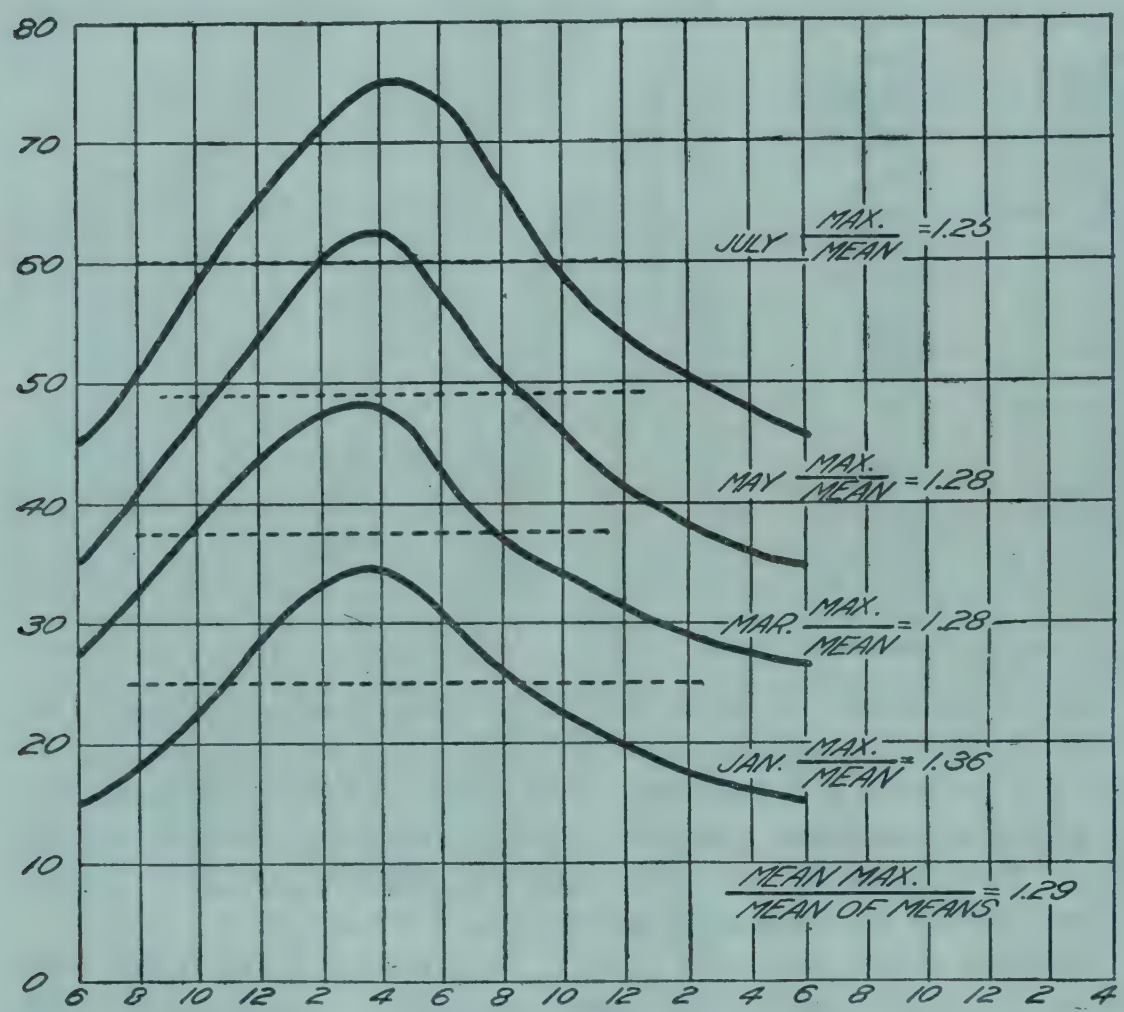

FIG. 3.—Average Utah thermograph records for various seasons.

coldest time is just before sunrise, instead of at midnight, as is sometimes thought. Heat is being received most rapidly at noon, but the temperature will continue to rise until the heat received and the heat lost per second are equal. As soon as the radiation rate exceeds the absorption rate the temperature will fall. Just after noon, even though the rate of absorption of heat is somewhat less than it was, yet it is still in excess of the radiation rate, and hence the net result is a rise in temperature. Inasmuch as this phenomenon is common to all localities, the thermograph records for clear days are very much alike in shape the country over. The convexity of the curve on the rising part or during the morning

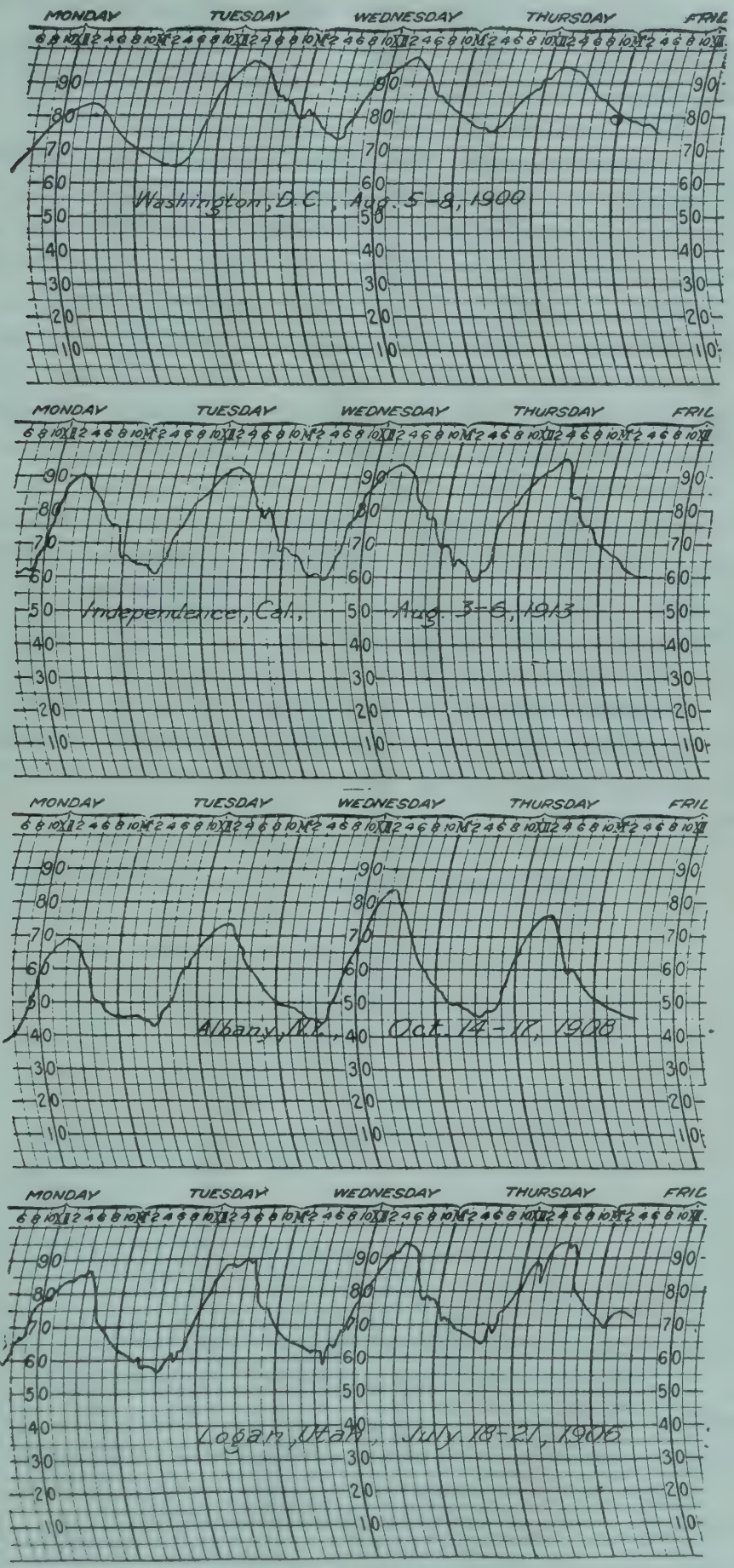

FIG. 4.—Actual thermograph records taken at widely separated stations in the United States.

hours and the concavity during the falling temperatures is mostly due to curvature of the vertical lines of the coordinate paper made necessary by the mechanism of the thermograph. The same temperature plotted on rectangular coordinate paper would make the curve approximately the shape of those of figure 1.

At first the writers thought that after having determined the mean daily temperature for the particular day they would determine the temperature for that particular hour by adding or subtracting a constant. The great change in the shape of the curve makes this method impracticable. However, it was found that if the temperature at a particular hour, such as the 3 a. m. value, is subtracted from the mean for the day and this answer divided by the mean for the day, the same answer will be obtained within less than 10 per cent no matter what day of the month or what month of the year is used. The variations are largely due to storms.

From a study of the temperature records of all the counties of Utah and for all months of the year the writers have determined the relation—expressed in percentage—of each hourly temperature to the mean daily temperature. These percentages of the mean for each of the 24 hours of the day are given in Table II. They come out practically constant for a particular hour of the 24, irrespective of the season, because as winter approaches and the mean daily temperature becomes lower the daily variation becomes less, the curve flattens (see fig. 3), and thus both values become less.

TABLE II.—*Relation of hourly temperatures to mean daily temperatures*

A. M.

Hour.	1	2	3	4	5	6	7	8	9	10	11	12
Per cent.....	85	82	79	76	73	71	72	73	77	87	97	108

P. M.

Hour.	1	2	3	4	5	6	7	8	9	10	11	12
Per cent.....	119	126	128	129	126	121	115	108	102	97	93	89

These percentages for the different hours are plotted in figure 5. The equation for this curve has been obtained by the method explained earlier and is as follows:

$$T = 97.33 - 9.8 \cos \theta + 0.88 \cos 2\theta - 0.52 \cos 3\theta - 23.26 \sin \theta + 3.52 \sin 2\theta - 1.42 \sin 3\theta + \text{----------} \quad (5),$$

or in terms of just one of the trinonometric functions:

$$T = 97.33 + 25.22 \cos (\theta - 67^\circ - 10') + 3.71 \cos 2(\theta - 37^\circ - 59') - 1.51 \cos 3(\theta - 23^\circ - 16') + \text{----------} \quad (6).$$

This result will be accurate for normal temperatures, and the weather forecast will help one to predict the actual temperatures, because it is abnormally warm just before and cold just after the passage of a cyclone.

A less scientific method but one requiring only arithmetic and a knowledge of the mean monthly temperature for the place will now be given for the solution of this problem.

From figure 2 it is seen that the curve is approximately flat at the top from July 15 to August 15 and at the bottom from December 15 to January 15. To get the mean daily temperature for any particular day of one of these months simply use the mean value given for the month. For other months of the year the mean daily temperature changes by one-third of 1° F. a day.

Suppose it is desired to determine the temperature at Ogden on September 21 at 1 p. m. In Table I it is seen that the mean September temperature for Ogden is 63° F. Since the twenty-first is six days removed from the fifteenth, multiply ⅓° by 6 and get 2°. Subtract this from 63°,

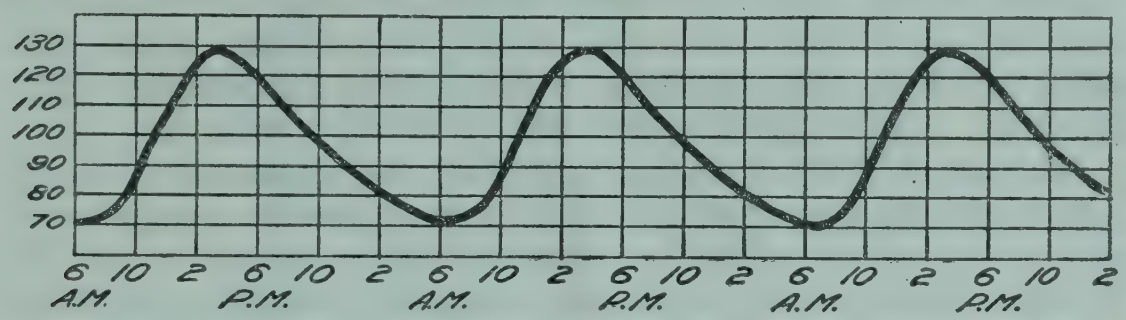

FIG. 5.—Hourly variation of temperatures expressed in percentages of the mean.

obtaining 61° as the probable mean temperature for the day. Turn now to Table II, where it is seen that the 3 p. m. temperature is 128 per cent of the mean. Hence multiply this percentage by 61° and get 78° as the probable temperature. This value could be improved much by getting the weather forecast from the Weather Bureau as explained earlier.

The first term of this equation is not 100 per cent, because the mean temperature of the day is not quite the average of the maximum and the minimum for the day.

To determine the temperature at 2 a. m., expressed as the percentage of the mean, divide 2 by 24 and multiply by 360°; 30° is obtained. Introduce 30 for θ in the above equation, and with the aid of a trigonometric table and a little multiplication the desired percentage will be obtained.

This equation will apply for any location where the mean daily temperature variation decreases as the mean daily temperature decreases, so as to give the ratio of the difference between the maximum and the mean to the mean a constant value of 1.29, as in Utah. The writers think it is of rather wide application.

The fact that this ratio comes out constant for all seasons is a coincidence, inasmuch as it holds only for the Fahrenheit scale of degrees and this scale was arbitrarily chosen.

The complete solution of the problem of determining the probable temperature at some particular place, such as Denver on June 15 at 2 p. m., would be to determine first the mean daily temperature for June 15 by means of equation 4, using as the first term of the equation the mean annual temperature for Denver and changing the date to degrees as explained earlier. By means of equation 6 just as it stands, change 2 p. m. to degrees and insert this value for θ in the equation and thus determine what percentage of the mean temperature the 2 p. m. temperature is. Multiply this percentage by the mean daily temperature obtained from the solution of equation 4 and get the desired 2 p. m. temperature.

The lowest monthly temperature given in Table I is for January at Uinta and is 16° F. With a daily variation of only 15°, a minimum of 8° would be expected. On certain days, however, negative temperatures are experienced. If the mean gets too low, some correction must be made, because the numerical solution does not, as it stands, allow negative values. When the mean temperature gets as low as 25° or lower add 20° to the mean, multiply by the percentage given in the table, and then substract the 20°; the normal temperature will be obtained. This will accommodate itself to negative values also.

The methods described above (particularly the trignonometric one) for determining the normal temperature give very accurate results for dry and humid regions. There are, however, two sources of error. The Weather Bureau record might not cover a long enough period of time, and the addition of another year's values might change the normal that we have used in making the equation. Also the equation, which is a series, might not include enough terms to have it represent exactly the normals. As explained earlier, these errors are very small.

Actual temperatures depart more from the normal in humid regions than in dry sections, and in comparing the normal as calculated by the foregoing methods with a particular observed temperature, the departure will be considerable in the humid section but only slight in dry regions. In the western part of the United States between the Rocky and the Sierra Nevada Mountains, an average yearly temperature departure exceeding 0.5° F. is unusual. In the average monthly temperatures, a departure from normal of 2° is common, but a departure of 4° is unusual. For the daily temperature, a departure of 4° from the normal is common, and a departure of 10° is unusual.

The States of Idaho, Nevada, Utah, Arizona, New Mexico, Montana, and Wyoming all have a relative humidity of about 50 per cent and an annual precipitation of from 10 to 20 inches—that is, the precipitation is light and the atmosphere comparatively clear. About 300 days out of 365, or about 80 per cent, are days free from rain, with the air clear and the sunshine bright most of the time. Better results, therefore, can be obtained in forecasting the temperature in this section of the country than in the sections where rains are frequent and the thermograph records are irregular in shape (see fig. 6).

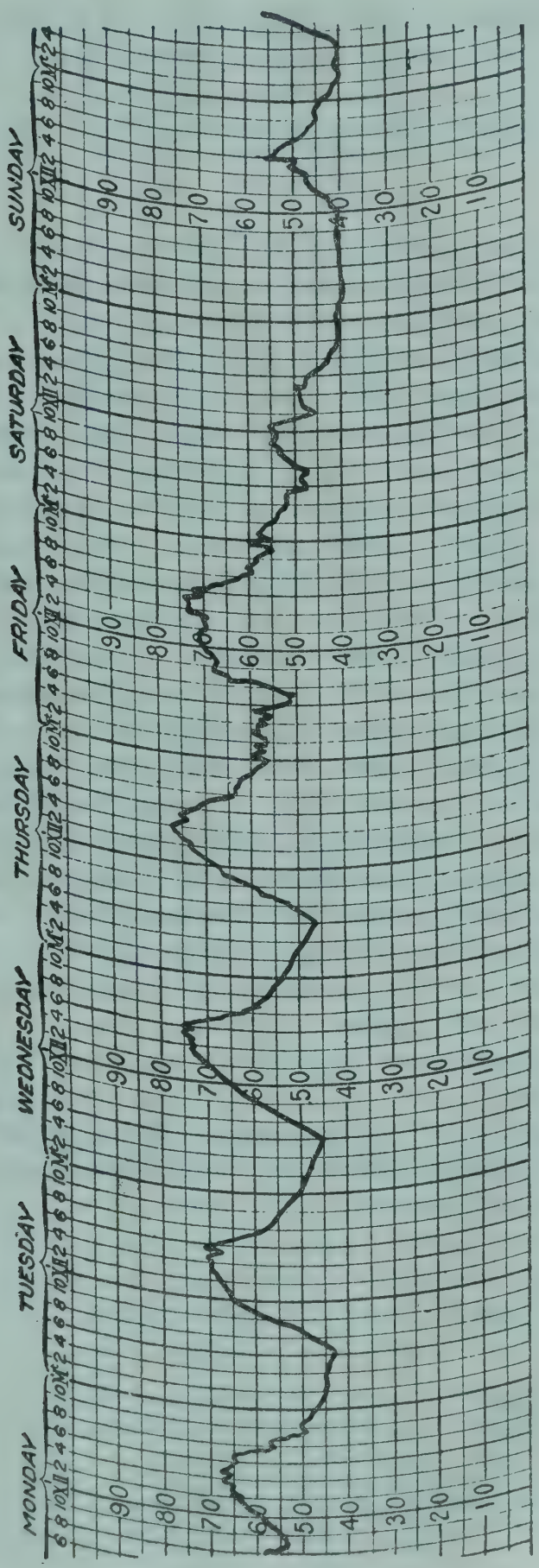

FIG. 6.—Effect of storm on diurnal variation of temperature.

Applying this rule and then checking the temperatures (not the normals) as they really occur, the writers found that the actual values differed from the theoretical value by from 1° to 15° F. with a mean departure of 7°. A very few errors were more than that. The chances in the arid West are 1 in 6 that the error will be less than 2°, 2 in 5 that the error will be 5°, 1 in 4 that it will be 10°, and 1 in 7 that it will be 15°.

SUMMARY

This paper gives an approximate solution of the problem of the determination of the normal temperature at a certain place at an assigned hour of the day on a particular day of the year.

An equation that shows the seasonal change in temperature is presented and gives the mean daily temperature in terms of the time of year. Another equation gives the percentage of the mean temperature that the temperature of a particular hour of the day is in terms of the time of day. The product of the results of the solution of the equations gives the temperature sought. The equation is of general application inasmuch as the first term is the mean annual temperature and the value for the location considered is to be inserted in the equation before using it.

An arithmetical solution is also presented. The hourly temperatures, expressed as percentages of the mean daily temperatures, are given. The mean monthly temperature for each of the 12 months must be known for the location considered. The mean daily temperature changes approximately one-third of 1° F. a day, except from December 15 to January 15 and from July 15 to August 15, when the mean temperature for any day is approximately equal to the mean temperature for the month With this information, the mean daily temperature is readily calculated, and by multiplying this value by the percentage found in the table for the particular hour considered, the desired probable temperature is obtained.

In the arid West the mean error of the method of determining the normal temperature is very small, but the mean error in predicting the actual temperature is 7° F., with 60 per cent of the errors less than this amount. These errors are due largely to the abnormal temperatures produced by rain and snowstorms.

TEMPERATURE RELATIONS OF CERTAIN POTATO-ROT AND WILT-PRODUCING FUNGI

By H. A. Edson, *Pathologist*, and M. Shapovalov, *Assistant Pathologist, Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Plant pathologists are well aware of the fact that certain parasitic organisms which seriously injure growing crops in one geographical latitude remain practically harmless in another. It seems reasonable to assume that the temperature of the different regions, aside from other climatic conditions, may greatly influence the occurrence of these parasites. As shown by Fawcett in his recent work,[1] a correlation exists between the cardinal temperatures of certain fungi in cultures and their geographical distribution and seasonal occurrence.

Moreover, the importance of properly regulated low temperatures for cold storage is fully recognized, not only for the purpose of controlling physiological and chemical changes, but also in order to check the progress of parasitic diseases. On the other hand, certain high temperatures are successfully employed not only to regulate ripening and sweating processes but also to effect the death of the invading parasites without lowering the vitality of the host.

There is, however, a regrettable lack of exact information regarding the temperature relations of different potato parasites. The following data secured in experiments with pure cultures of some of the most common potato-rot and wilt fungi may prove to be interesting and significant. They explain to a certain degree the predominance of these organisms in definite regions and definite seasons. They also permit certain practical conclusions regarding the temperatures which may control or eliminate these fungi.

CULTURES AND METHODS EMPLOYED

The cultures used in the tests with statements concerning their origin and time of isolation are listed below. As will be seen later from the graphs, the two strains of Verticillium, one from the South and the other from the North, showed distinctly different thermal behavior. Only one strain of each species of Fusarium was used in all experiments.

Fusarium coeruleum (Lib.) Sacc. (Culture No. 201).[2] Isolated by C. W. Carpenter in March, 1915, from potato tubers grown in the State of New York.

[1] Fawcett, H. S., preliminary note on the relation of temperature to the growth of certain parasitic fungi in cultures. *In* Johns Hopkins Univ. Circ. 293 (n. s. no. 3) p. 193–194. 1917.

[2] Numbers accompanying each culture refer to the writers' catalogue.

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F. discolor, var. *sulphureum* (Schlecht) App. and Wollenw. (Culture No. 203). Dr. Wollenweber's culture isolated at Dahlem, Berlin, in June, 1909.

F. eumartii Carp. (Culture No. 204). Isolated by C. W. Carpenter in January, 1914, from a stem-end dry-rotting tuber grown in Pennsylvania.

F. oxysporum Schlecht. (Culture No. 208). Isolated by H. A. Edson in October, 1916, from potato tubers grown in New Jersey.

F. radicicola Wollenw. (Culture No. 211). Isolated by H. G. MacMillan in October, 1916, from potato tubers grown in Colorado.

F. trichothecioides Wollenw. (Culture No. 214). Isolated by O. A. Pratt in October 1916, from potato tubers grown in Idaho.

Verticillium albo-atrum Reinke and Berthold. (Culture No. 426). Isolated by C. W. Carpenter in September, 1915, from an eggplant grown at Shepherdstown, W. Va.

V. albo-atrum (Culture No. 427). Isolated by M. Shapovalov in September, 1917, from the vascular system of a wilted potato stem grown at Presque Isle, Me.

The growth of these fungi at various temperatures was studied in plates containing 10 cc. of a 2 per cent potato agar without sugar. A small drop of a water spore suspension of the respective fungus was placed in the center of each plate by means of a 2-mm. loop. The plates were then distributed in the incubators running at from 1° to 40° C., with approximately 5° difference between chambers. Additional sets of plates of *Fusarium oxysporum* and *F. radicicola* were kept at 37° and 39°, respectively, the maximum temperature here being of particular interest on account of a thermophylic habit of these organisms. Observations were made and measurements of the diameters of the colonies were taken daily for a period of two weeks. At the end of the first week the most rapidly growing colonies reached the edges of the plates; therefore further data were of little comparative value and were omitted in the final compilation. The tests were repeated three times in their entirety and in some inconclusive cases four and five times. The average results then were plotted and are presented in the accompanying graphs.

DISCUSSION OF RESULTS

A general examination of the figures 1 to 8, which represent daily accumulations of growth, at once reveals a peculiar common feature in the structure of the graphs: each series of curves characterizing the growth of a given organism originates at scattered points to the left, the lowest thermal points, then rises in the direction of the optimum temperature, and finally falls to a single point at the right, the highest thermal limit. *Fusarium oxysporum* and *Verticillium albo-atrum* No. 426 appear to be slight exceptions to this rule, but only for the first day. Quite a contrasting picture is seen in figure 9, where each curve shows the amount of growth produced by a corresponding organism for the total period of 7 days. Here, with but one exception, the entire series of curves has its origin at a single point to the left and is distributed at different points to the right. Thus, it is evident that the highest thermal point of growth was reached within the first 24 to 48 hours, while the lowest limit did not become apparent until the expiration of from 5 to 7 days.

The minimum temperature of growth and germination for the five Fusaria and the two Verticillia lies either somewhat above or somewhat below 5° C. *Fusarium discolor* var. *sulphureum* formed a small amount of visible growth at 5° on the sixth day, but neither growth nor germination could be seen at 1° after 7 days, although it was observed after 2 weeks. *F. oxysporum* did not grow and did not germinate at 5° even in two weeks. The remaining fungi did not produce visible growth at 5° in the first 7 days, but the germination was found to have taken place

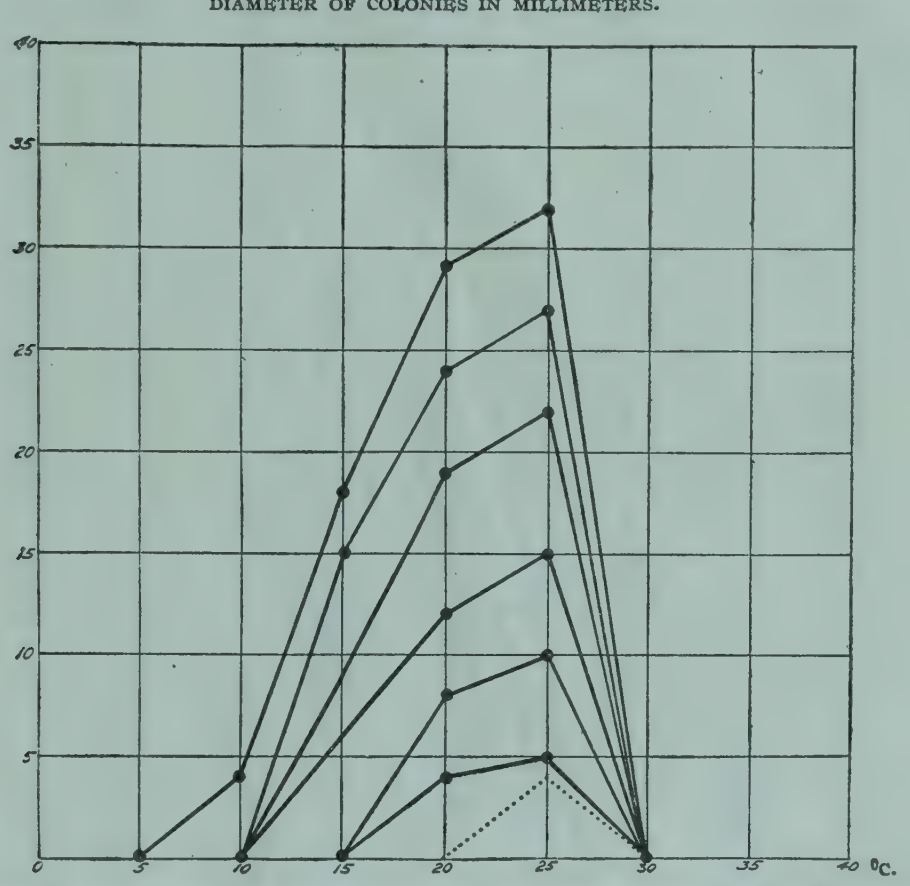

FIG. 1.—Graph showing the rate of growth of *Fusarium coeruleum* on potato agar at different temperatures. The growth made during the first 24 hours is marked here with a dotted line. It was very indistinct because the fungus was not in high culture.

when plates were examined under the microscope. The length of the germ tubes, however, varied considerably with different organisms. This difference was particularly significant in the two Verticillia; the spores of the culture No. 426 produced only very short germ tubes, while the spores of the culture No. 427 formed a fair weave of fungal threads. Brooks and Cooley [1] report no germination with *F. radicicola* at 5° even after the expiration of 10 days, but they made their studies with cornmeal agar cultures and the results thus obtained may not be fully comparable with those here reported.

[1] BROOKS, Charles, and COOLEY, J. S. TEMPERATURE RELATIONS OF APPLE-ROT FUNGI. *In* Jour. Agr. Research, v. 8, no. 4, p. 139–164, 25 fig., 3 pl. 1917.

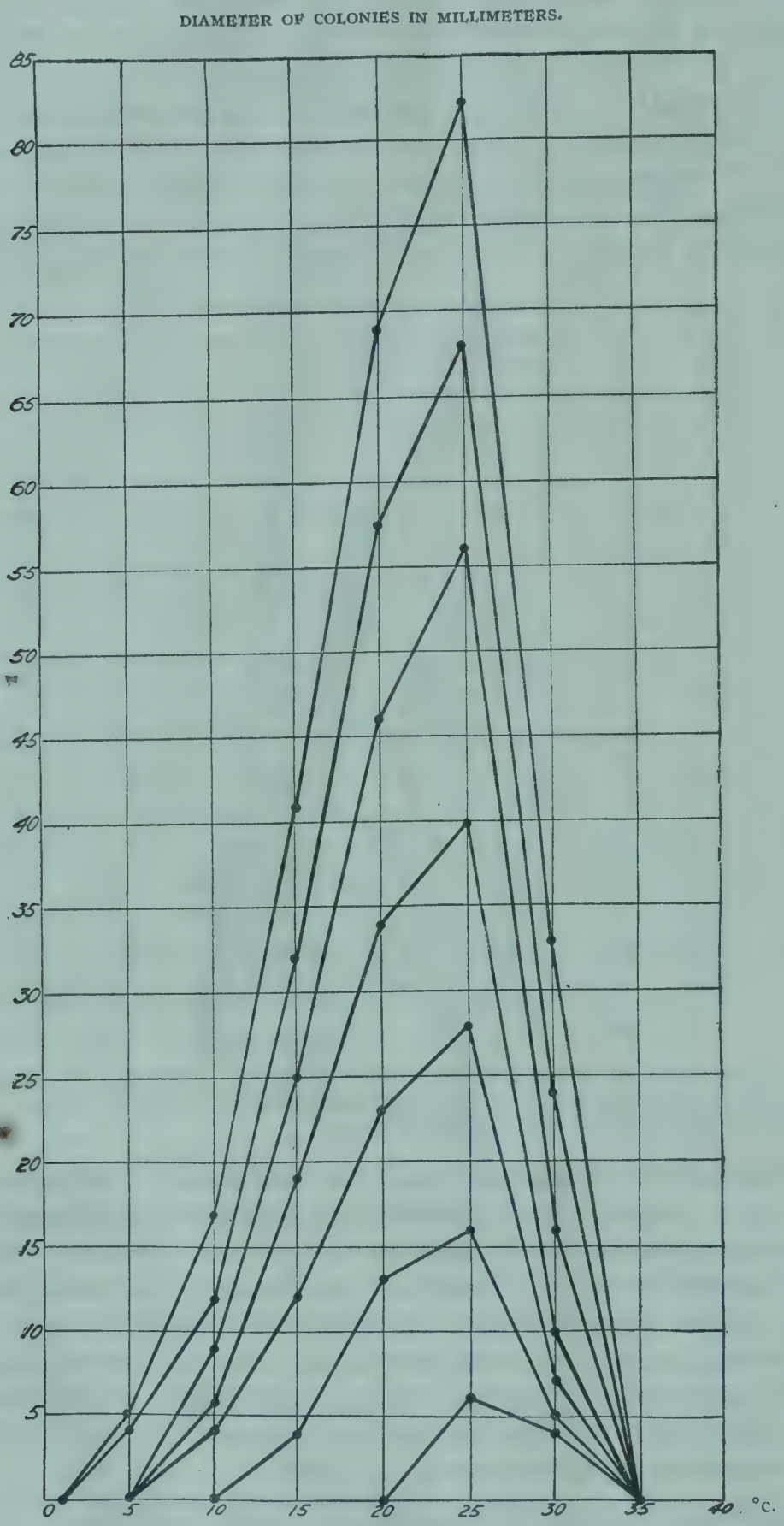

FIG. 2.—Graph showing the rate of growth of *Fusarium discolor* var. *sulphureum* on potato agar at different temperatures.

The optimum temperature divides the potato fungi into two groups. One, consisting of four Fusaria and two Verticillia, has its optimum in the neighborhood of 25° C.; and the other, comprised of *F. oxysporum* and *F. radicicola*, has its optimum in the vicinity of 30°. As shown by Brooks and Cooley,[1] the optimum temperature for *F. radicicola* on corn-meal agar is the same as stated here for cultures on potato agar—that is, 30°. After 7 days of incubation at this temperature the two latter parasites practically covered the plates. *F. discolor* var. *sulphureum* and *F. trichothecioides* produced the next largest amounts of growth, and the

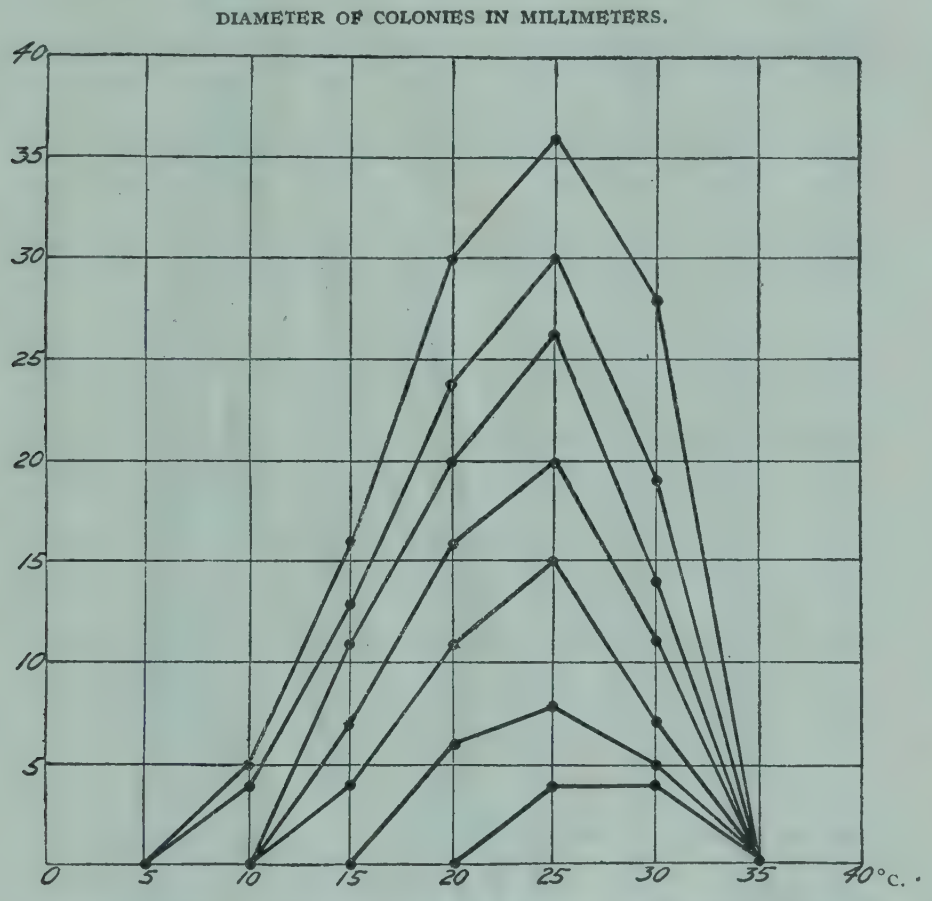

FIG. 3.—Graph showing the rate of growth of *Fusarium eumartii* on potato agar at different temperatures

remainder formed colonies measuring on the average only 22 to 32 mm. in diameter.

The maximum temperature at which growth occurred in *Fusarium coeruleum*, *F. trichothecioides*, and *Verticillium albo-atrum* No. 427 was at or slightly below 30° C. Germination took place with *F. trichothecioides* at 30°, but no growth visible to the unaided eye was formed. The other two fungi did not even germinate at this temperature. *F. discolor* var. *sulphureum*, *F. eumartii*, and *V. albo-atrum* No. 426 were not able to germinate at 35°. The Verticillium spores remained unchanged, but the normal typical spores of the two Fusaria were gradually changed to chlamydospores. Similar transformation of the spores of *F. oxysporum* occurred at 37° and of *F. radicicola* at 39°.

[1] BROOKS, Charles, and COOLEY, J. S. Op. cit., p. 156.

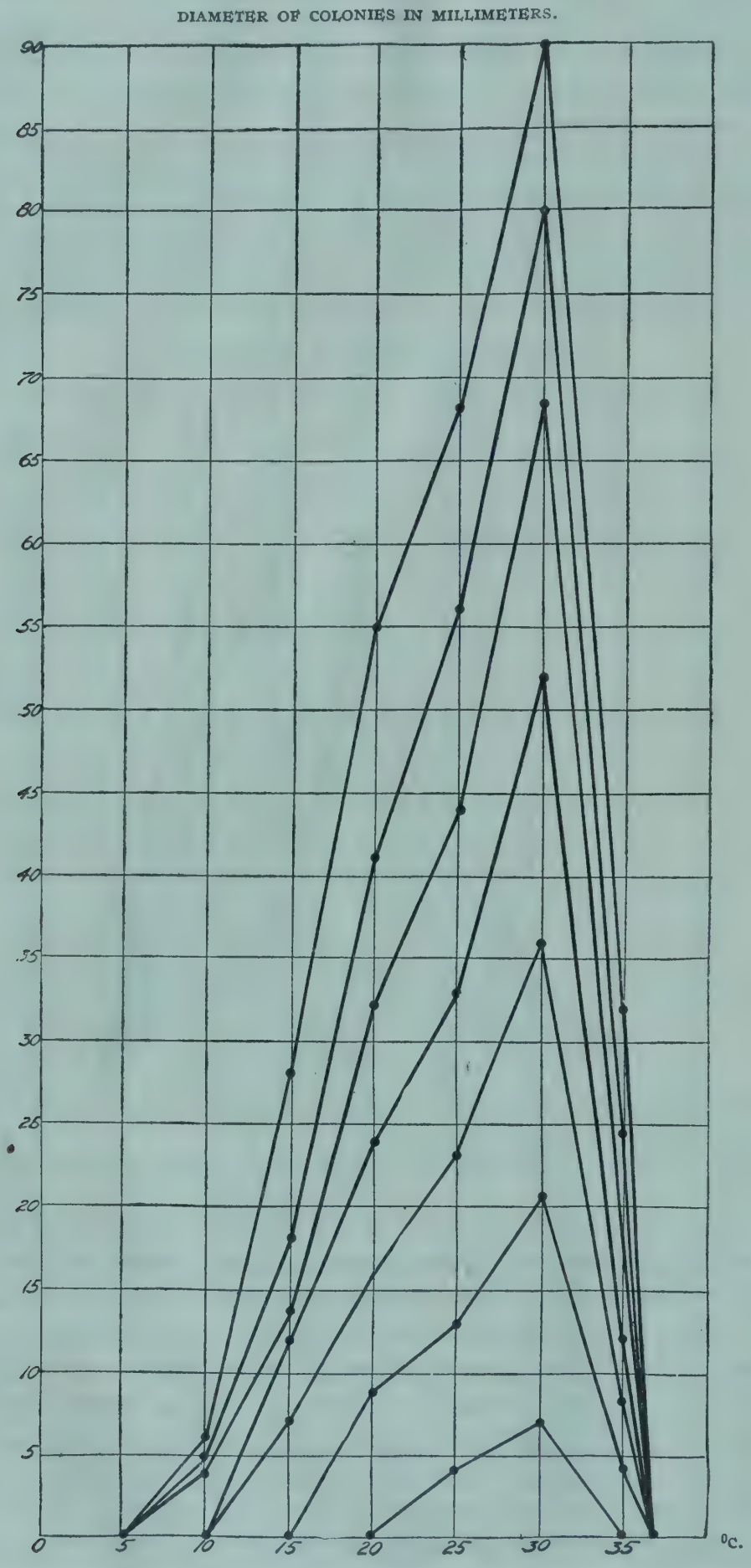

FIG. 4.—Graph showing the rate of growth of *Fusarium oxysporum* on potato agar at different temperatures.

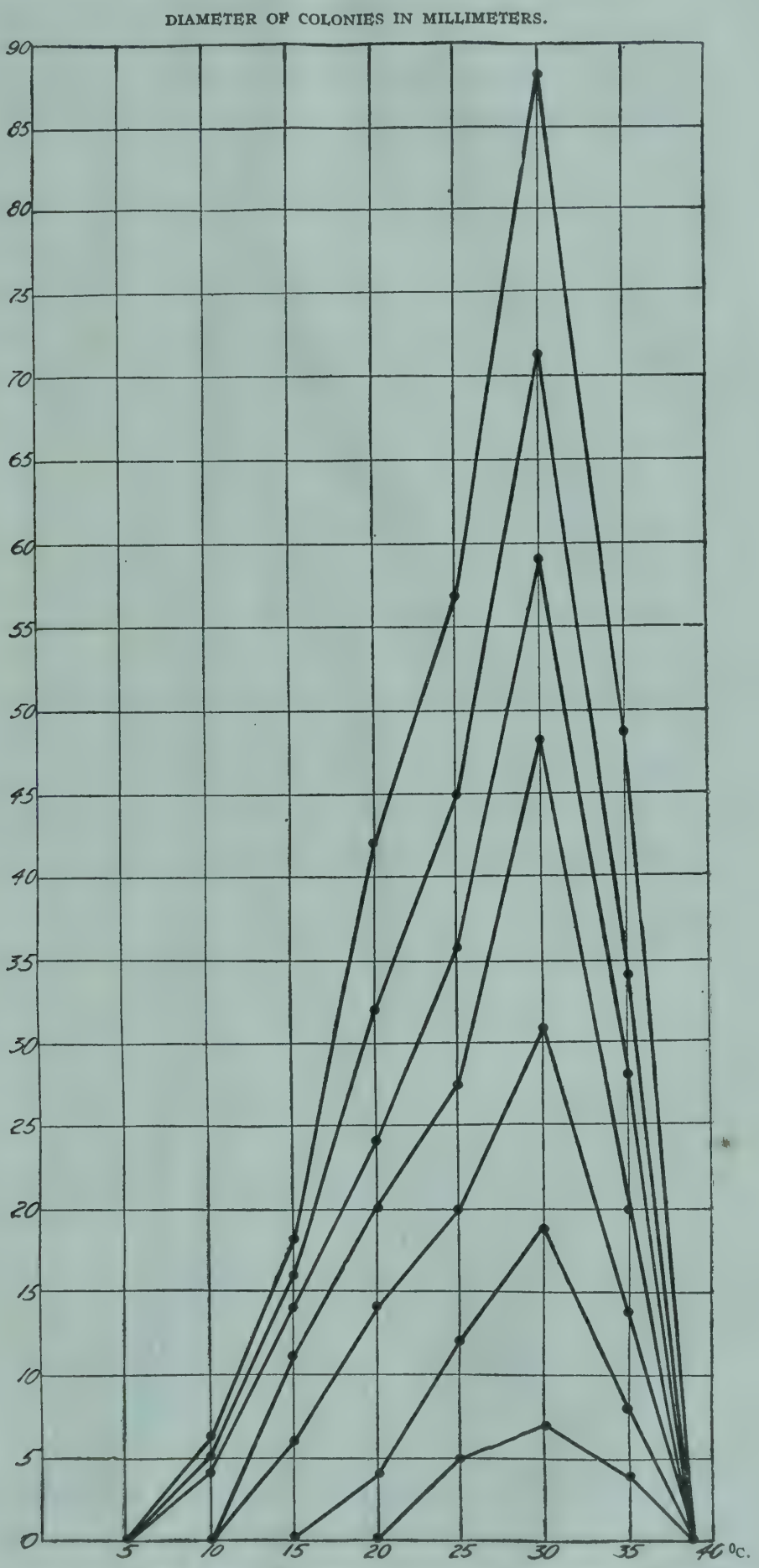

FIG. 5.—Graph showing the rate of growth of *Fusarium radicicola* on potato agar at different temperatures.

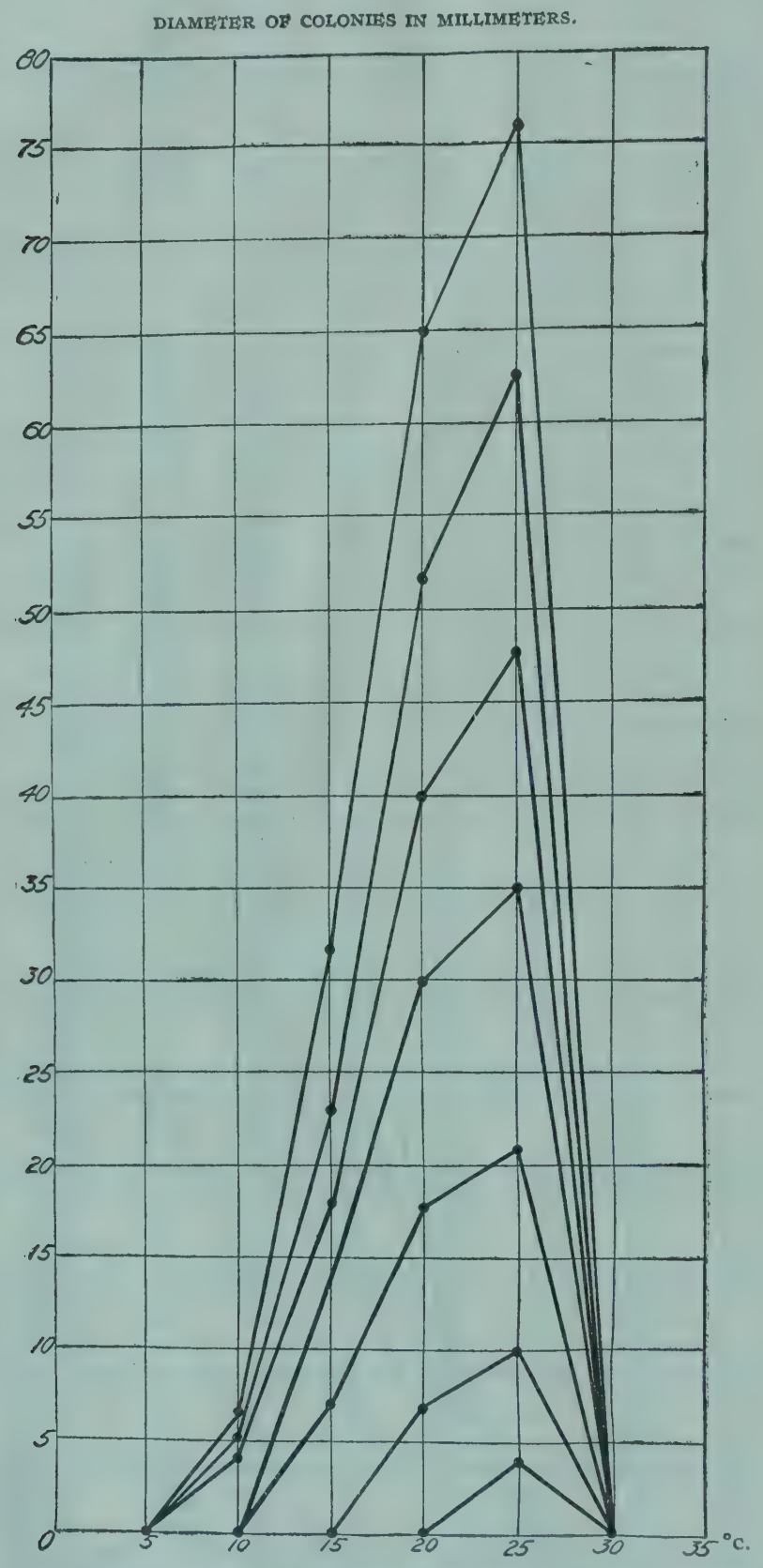

FIG. 6.—Graph showing the rate of growth of *Fusarium trichothecioides* on potato agar at different temperatures.

The initial growth after the first 24 hours was noted always either at the optimum point alone or at the optimum point and at one or two lower

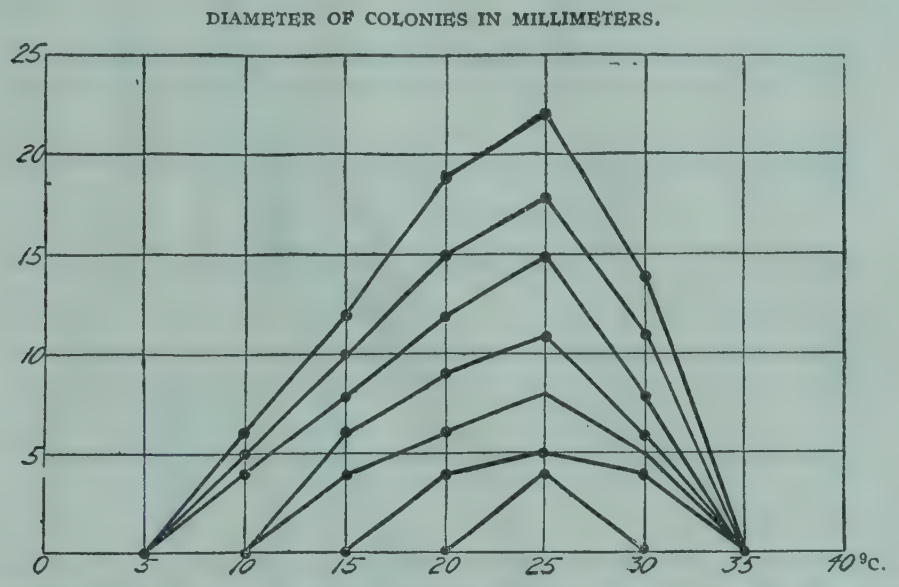

FIG. 7.—Graph showing the rate of growth of *Verticillium albo-atrum* No. 426 on potato agar at different temperatures.

or higher grades of temperature, but never exclusively at any point other that the optimum temperature (fig. 1–8).

Comparing the foregoing results of the temperature studies with the geographical distribution of the fungi, we find that the parasites prevailing

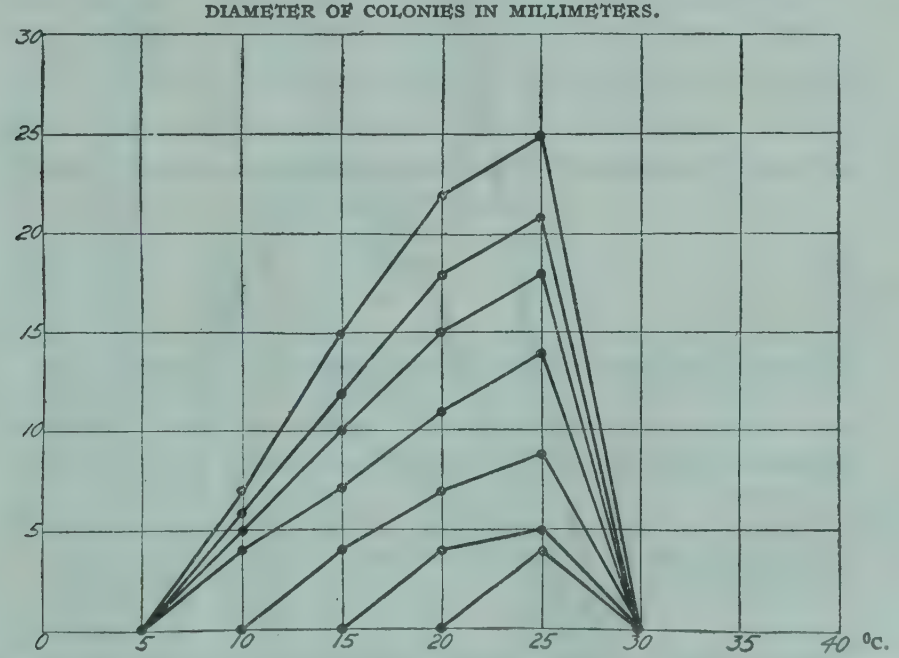

FIG. 8.—Graph showing the rate of growth of *Verticillium albo-atrum* No. 427 on potato agar at different temperatures.

in northern sections of the country (*Fusarium coeruleum* and *Verticillium albo-atrum* No. 427) have comparatively low maximum and low optimum points; those from central zones (*F. eumartii* and *V. albo-atrum* No. 426) exhibit a higher maximum, although they still retain practically the same

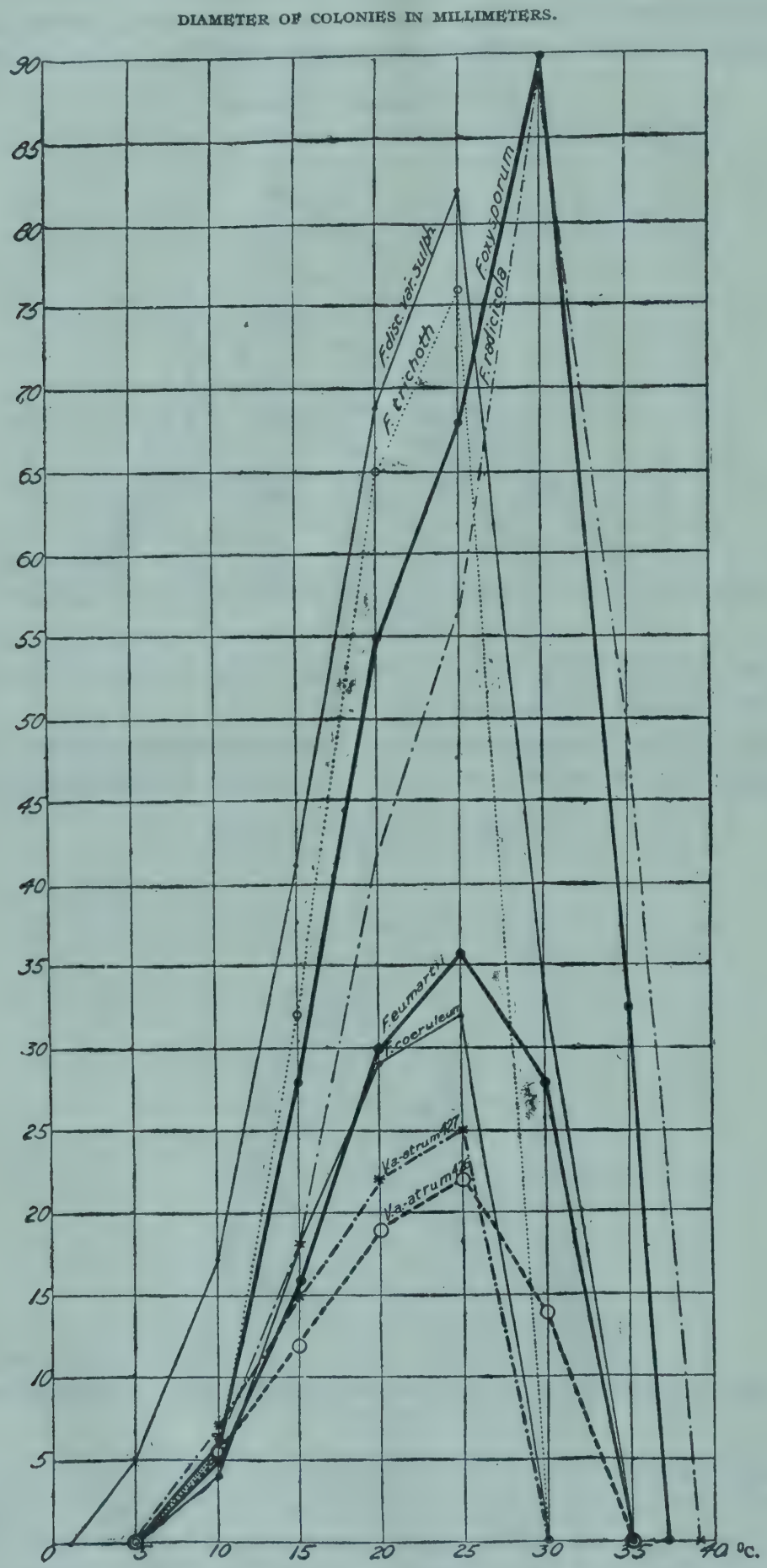

FIG. 9.—Graph showing the total amounts of growth produced by different potato-rot and wilt fungi during the first seven days on potato agar at different temperatures.

moderate optimum; while the parasites of the southern zones (*F. radicicola* and *F. oxysporum*) shift very decidedly toward both a higher optimum and a higher maximum temperature.[1] *F. discolor* var. *sulphureum* occupies a somewhat intermediate position between these groups, which may well explain its ubiquitous nature as a storage-rot organism. It has a comparatively wide thermal range of growth with a moderate optimum temperature. *F. trichothecioides* should be referred to the northern group of the fungi, taking as a basis its thermal behavior in artificial cultures. It differs, however, from the other two fungi of this group by its more rapid growth at and somewhat below the optimum temperature. This corresponds to the very active destruction caused by this fungus after it becomes well established in the host tissues. The two Verticillia give a particularly significant example of the correlation between the thermal response of the fungi and their geographical occurrence. The Northern strain shows a better adaptation to lower temperatures and does not grow above 30° C., while the southern strain shows a better adaptation to higher temperatures and gives a fair growth at 30°. Their morphological differences have not been sufficiently studied to warrant their separation as two different species. It may be stated, however, that they are not fully identical in pure cultures. Culture No. 426 has a tendency to produce numerous small sclerotia, which are practically absent in culture No. 427. The difference in response between the two wilt-producing fungi, *F. oxysporum* and *V. albo-atrum* No. 427, both with regard to their lower and higher growth limits and their optimum points, was even greater than with the two Verticillia. The results correlate with their geographical distribution and explain why *F. oxysporum*, although present in the soils of Maine, as well as on decaying tubers and in stem lesions of the potato there, has, nevertheless, not been associated with potato-wilt in that region.

A correlation can be drawn also between the temperature relations and the seasonal occurrence of *Fusarium oxysporum* and *Verticillium albo-atrum* No. 427, as shown by the following experiments conducted at Arlington Farm, Va. A certain number of tubers of the Irish Cobbler variety were divided into three groups. One group was inoculated with *F. oxysporum*, one with *V. albo-atrum*, and one was left uninoculated for control. They were planted on the farm plots on April 27. A second planting of similar groups was made on July 17. The first crop was dug on August 26 and the second crop on October 21. Immediately after digging the tubers were examined and wherever the stem-end discoloration was present, isolations were made. The results of these isolations are given in Table I.

[1] The zones referred to should not be regarded as definitely limited geographical areas. The terms employed are relative only, and the areas overlap. They are determined not only by latitude but by elevation and by soil climate. The fungi assigned to one zone may and usually do occur in the other. The assignment has been determined largely by the relative prevalence and parasitic virulence of the organisms.

TABLE I.—*Seasonal prevalence of Fusarium oxysporum and Verticillium albo-atrum under the climatic conditions of the vicinity of Washington, D. C.*

Treatment of seed tubers.	Early planting.						Late planting.					
	Total number of tubers.	Tubers showing discoloration.	Tubers giving cultures of—			Number sterile.	Total number of tubers.	Tubers showing discoloration.	Tubers giving cultures of—			Number sterile.
			F. oxysporum.	*V. albo-atrum.*	Miscellaneous fungi.				*F. oxysporum.*	*V. albo-atrum.*	Miscellaneous fungi.	
Inoculated with *F. oxysporum* a few days before planting	60	11	8	0	0	3	183	10	2	0	0	8
Inoculated with *V. albo-atrum* a few days before planting	53	30	3	1	8	18	159	44	3	7	2	29
Control tubers, uninoculated	46	18	8	0	0	10	119	16	1	0	0	15

It appears from this table that there were more tubers infected with *Fusarium oxysporum* in the early crop grown at higher temperature than in the late crop grown at lower temperature, and vice versa with *Verticillium albo-atrum.* The presence of the Fusarium infection in the control tubers indicates that, probably, the largest part of it came from the soil, while the infection of the new tubers with Verticillium came exclusively from the seed. Verticillium was absent both in the control plot and in the Fusarium plot of each crop.

The fact that the growth of potato fungi was seriously inhibited at or somewhat below 5° C. is of a considerable practical importance. Although the curves showing the temperature relations of these fungi in pure cultures may not always coincide with those which would designate their development in the host tissues, yet one may be reasonably certain that a temperature of about 40° F. or slightly below will suffice to check the spread of the Fusarium potato tuber-rots in storage. Brooks and Cooley's studies[1] show that the growth of apple-rot fungi is retarded by low temperatures to a much greater degree on the host itself than on artificial media The requisite temperature for successful infection is, therefore, higher than the minimum temperature necessary for growth in cultures.

High temperature treatment of the seed tubers to effect the death of invading parasites suggests itself as a thing which may deserve certain attention. In view of the thermophylic habit of *Fusarium oxysporum*, the possibility of application of sufficiently high temperature to cause the death of the parasite and not to injure the vitality of the tuber is quite remote. The case with *Verticillium albo-atrum* is, however, more hopeful. Certain preliminary experiments conducted by the writers

[1] BROOKS, Charles, and COOLEY, J. S. OP. CIT.

indicate that while if sufficient moisture is provided the spores and the mycelium of this organism remain alive at 30° C., they do not survive exposure for several days to a temperature of 35° or higher.

Several potato agar plates inoculated in the manner described above were kept at 30° and 35° C. for 7, 8, 11, and 14 days and then removed to room temperature. No growth ensued in any of these plates.

Nine beef-broth tubes were inoculated heavily with the spores of Verticillium and placed in incubators set at 25°, 30°, and 35° C. At the end of two days spores germinated at the two lower temperatures and formed growth visible to the unaided eye. Spores at 35° remained unchanged; but when one tube was brought to the laboratory, germination took place and growth developed. Two additional tube cultures were kept at the original temperature two weeks and then removed to the laboratory. No growth resulted in these cultures.

Thirty-five tubers selected in the field from hills badly affected with Verticillium-wilt in northern Maine were incubated at 25°, 30°, 35°, 37°, and 41° C., with 7 tubers to each compartment. After 13 days 2 tubers were taken from each lot, and cultures were made from the discolored stem ends. Twelve plantings from the material kept at the two lower temperatures yielded 5 cultures of *V. albo-atrum* while 7 remained sterile. No culture was obtained from material exposed to 35° or higher. Two of the remaining tubers of each lot were incubated 22 days longer, or for a total period of 35 days. Isolations from the 25° and 30° lots gave 6 cultures of Verticillium out of 12 plantings. The lots at 35° and 37° yielded sterile cultures. The tubers held at 41° were dead and blackened and were discarded. The remaining 3 tubers of each lot were kept for an additional 11 days. Isolations were made from the material held at 30°, 35°, and 37°. Three out of 9 plantings from the 30° material gave cultures of *V. albo-atrum*, while plantings from the tubers stored at higher temperatures proved sterile. Thus, the results were uniformly identical; the fungus survived exposure to 30°, but in no single case survived the exposure to 35°.

Incubation of freshly prepared culture plates at a properly chosen high temperature may serve as a useful practical method for the differentiation of the fungi which possess strikingly different maximum temperatures but which are otherwise very similar in cultural characteristics, particularly if they do not readily yield high cultures. Such is the case with *Fusarium coeruleum* and *F. radicicola*. They resemble each other very closely in color reactions on standard culture media, yet it usually requires a considerable length of time before typical spores of the former can be secured. If plate cultures placed at 35° C. for two or three days show no growth whatever, there is perfectly safe ground to assume that the fungus is not *F. radicicola*. This test may be very helpful when a prompt identification is desired or when additional evidence to support a conclusion is being sought.

CONCLUSIONS

(1) A certain degree of correlation exists between the temperature relations of some potato fungi in pure cultures and their geographical distribution and seasonal occurrence. The correlation is particularly striking in the wilt-producing fungi, *Fusarium oxysporum* and *Verticillium albo-atrum*.

(2) A temperature of about 40° F. should hold Fusarium tuber rots in check during storage.

(3) The susceptibility of *Verticillium albo-atrum* to high temperatures suggests the possibility of a heat treatment for infected seed tubers.

(4) Temperature tests in certain cases may serve as a useful supplementary method for the identification of fungi exhibiting contrasting thermal relationships.

GERMINATION OF BARLEY POLLEN

By STEPHEN ANTHONY, *formerly Assistant*, and HARRY V. HARLAN, *Agronomist in Barley Investigations, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The artificial germination of plant pollens has met with varying success. As a whole, the pollen of the Gramineae has proved very difficult to germinate. While experiments with the pollen of many plants have resulted not only in ready germination but in satisfactory methods of preserving the pollen for considerable periods of time, the results with many of the Gramineae have been far from satisfactory. This is especially true of self-fertilized forms, for many of which no artificial germination of pollen has been secured. The pollen of corn, on the other hand, was germinated readily by Andronescu (*1*).[1] It is believed that the pollen studies reported herein record the first artificial germination of barley pollen.

These studies grew out of a series of observations made by the junior author on the lateral florets of 2-row barley. The stamens of these florets are sometimes abortive, sometimes rudimentary, and sometimes well developed, producing abundant pollen. In an attempt to classify these variations, the question naturally arose as to whether or not this pollen was viable in all cases, and for such determination a reliable method of artificial germination obviously was desirable. It was to fill this need that the investigation was undertaken. The senior author was associated in barley investigations at that time, and the original project was intended to be a joint study. As it turned out, however, practically all of the laboratory studies were made by the senior author, while the field experiments were contributed by the junior author.

REVIEW OF THE LITERATURE

The volume of literature on the germination of pollen is so extensive that mention of only those papers having a special significance in their relation to the present studies will be included. These, for the most part, are cited at specific points in the text. According to older views, pollen fell into three classes: (1) pollen for which water alone was necessary for germination, (2) pollen which required, besides water, a chemical stimulant, (3) pollen which germinated in a solution of sugar of various concentrations. Mohl (*8*) in 1834 germinated pollen in water. Van

[1] Reference is made by number (italic) to "Literature cited," p. 535-536.

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Tieghem in 1869 (*11*) used an artificial medium. Jost (*2*), studying the physiology of pollen, concluded, from the fact that it germinates on the stigmas of other plants, that physical factors and not specific substances of the stigma were responsible for germination.

Jost (*3*), working with the pollen of Glyceria, Dactylis, and Secale, accidentally noticed in one of his experiments that pollen of these plants would germinate in close proximity to a drop of water. He was able thus to germinate the pollen of rye (*Secale cereale*) without any medium whatsoever.

Mangin (*7*) concluded that pollen of certain plants could germinate and grow from the food reserves within the pollen grain, while pollen of other plants did not grow well except in the presence of external nutrients.

EXPERIMENTS WITH SOLUTIONS

In the experiments of the authors the first trials of germination were made in water and solutions of sugar, agar, and other nutritive substances of various osmotic concentrations. It is unnecessary to report more of the aqueous tests than the behavior in sugar solutions. High concentrations resulted in plasmolysis. Low concentrations resulted in bursting in mature pollen. Immature pollen grains increased in size but did not burst. Mature pollen did not increase in size before bursting. In concentrations slightly less dense than those at which plasmolysis begins, small knobs were formed by the protrusion of the cell contents from the pore through which germination takes place. These knobs reached a length of from 2 to 4 μ but did not grow further. The knobs frequently were distended by continued absorption of water. If the water absorption is rapid, the bulging intine, which surrounds the extended cell contents, is ruptured before the knobs attain much size. Various stages of knob formation are shown in Plate 60.

EXPERIMENTS WITH MOIST CHAMBERS

When it became evident that germination was not likely to be secured in solutions, trials were made on various membranes of plant and animal origin. These experiments met with no success. Trials were made in moist chambers with and without membranes. The moisture in these chambers was supplied sometimes by drops of free water and sometimes by fragments of living plant tissue, the necessary humidity in the latter case arising from the evaporation from ruptured cells. No germination was obtained. If the cells became too moist, the pollen burst; if they became too dry, the pollen shrank.

The water adjustment of the pollen was so delicate that it seemed impossible to obtain a method of sufficient refinement to secure and maintain the proper conditions. If was found that the pollen on a slide could be readily killed, drowned as it were, by blowing one's breath upon it. The final success came from an observation the senior author made at Aber-

deen, Idaho, on opening flowers early in the morning, about the time when fertilization is most frequent. The stigmas, when viewed with a lens were seen to be covered with minute droplets of water, apparently condensed there by evaporation from other tissues more exposed to external heat. This observation led to the belief that germination was dependent purely upon physical factors and that the control of moisture was essential to artificial germination.

The conditions observed in the field were duplicated as nearly as possible in the laboratory. Pollen was taken from an anther ready to burst and dusted on a slide inside a loosely placed Van Tieghem cell. A piece of mesophyll from the leaf of the garden pea was placed in the cell to supply water. The cell was covered with a cover glass and the slide placed outside on the window ledge. The idea in this procedure was that as the condensation of the moisture progressed there would be a slow transition from a very low humidity to a very high one and eventually to the deposit of free water on the pollen. At some point between these extremes, conditions favorable to the growth of pollen would be met. Germination was secured inside of five minutes on the first attempt.

Later trials frequently resulted in large percentages of germination. Germination was accomplished both with mesophyll from green leaves and with free drops of water as sources of humidity.

Uncovering the moist chamber invariably led to failure; the knobs which had formed never attained any size, and the pollen subsequently died. When the pollen was left undisturbed for five minutes, examination showed germination amounting to 40 per cent. The tubes attained a length of from 60 to 100μ. No bursting of any tubes was noticed. The rest of the pollen was either starting to collapse or was in a normal state, apparently viable but not germinating. It was noticed that if the slide was resting on a cool medium—that is, a moistened filter paper—the pollen grains were swollen, and bursting was pronounced. The condensed droplets of moisture could be seen easily around the pollen grains, directly drowning them.

However, difficulties of germination did not disappear with a realization of the conditions necessary to secure growth, and the authors have not been able to bring all the factors under absolute control. The proper range of humidity must coincide with a certain range of temperature. The moisture supply must be ample to secure germination and yet the transition must be slow enough so that germination will be accomplished before the pollen is drowned. The delicacy of the water adjustment is difficult to realize. Plate 61, A, shows normal pollen grains and Plate 61, B, the same grains exposed for 2 minutes to the air. Some of the shrunken grains may still germinate; but with the loss of very little more water, germination becomes impossible. Perfectly normal pollen of tested germination was left uncovered on a dry slide for 10 minutes at a temperature of 20° C. The authors were unable to germinate

this pollen, either by artificial means or on the stigma. On the other hand, normal pollen grains will burst from too rapid and too extensive water absorption if the water condensation is too rapid. Weather conditions affect the growth of pollen both in the field and in the laboratory. Germination is accomplished with difficulty on cold, wet days. There is very little fertilization in the field under such conditions; and germination in the laboratory on such days is obtained with difficulty, because the necessary conditions are less easily secured and viable pollen is much harder to obtain.

FERTILIZATION IN THE FIELD

In order to correlate the laboratory experiments with fertilization under field conditions, extensive experiments were made by the junior author in 1917 and 1918. Both the period of receptivity of the stigma and the duration of viability of the pollen were studied. In the study of the receptivity of the stigma the results were strikingly uniform. Two hundred and eighty flowers were emasculated in one afternoon. For this purpose spikes were chosen which, by the length of the protruding awn and the first suggestion of the parting of the leaf sheath to release the side of the spike, indicated that fertilization would have occurred the following day. Forty flowers were pollinated immediately upon emasculation. Forty were pollinated on each succeeding day for six days. The results are shown in figure 1.

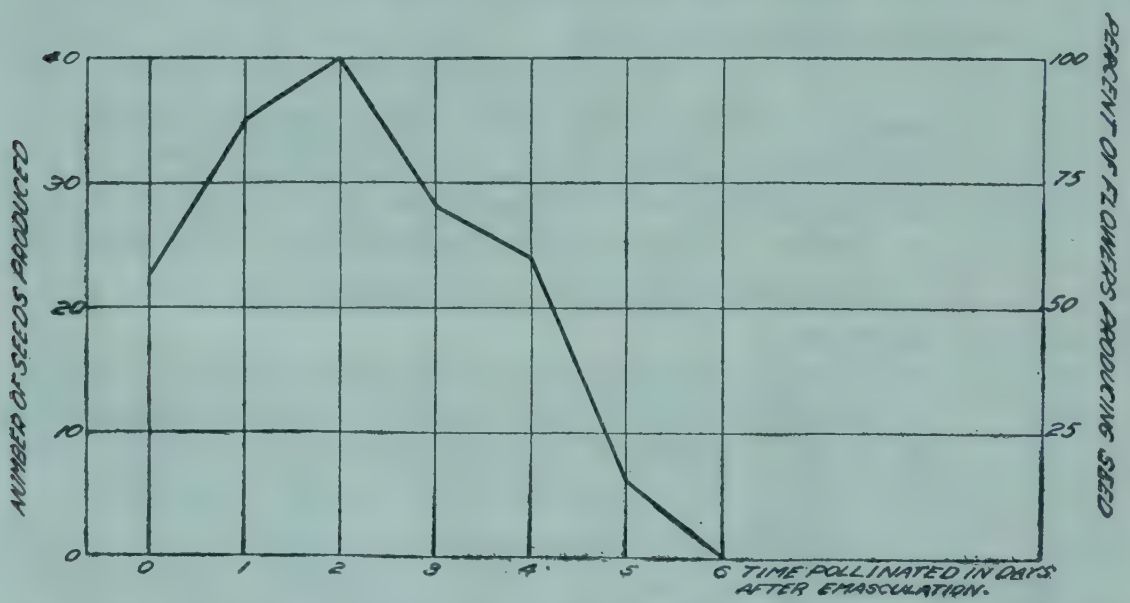

FIG. 1.—Period of receptivity of the stigma under field conditions as shown by the number of seed produced when 40 flowers were pollinated on emasculation and the same number on six successive days after emasculation.

The percentage of successful pollinations increased for two days. Of those pollinated two days after emasculation, 100 per cent of the ovaries set seed. From this time there was a gradual decrease, until on the sixth day no pollinations were successful. It is obvious that failures in

hybridization are due much more to faulty pollen than to any lack of receptivity of the stigma.

The viability of the pollen was tested both in 1917 and in 1918. As in the laboratory, the results were inconsistent, the variations indicating the readiness with which the viability of the pollen is affected. Forty flowers were used as a unit, as before, and all pollinations were made 48 hours after emasculation. In 1917, a different variety was selected for the test than was used in the experiment on receptivity of the stigma. Owing to the more advanced stage of the development of the plants, pollen was more difficult to obtain, and the highest percentage of successful pollinations was lower than in the previous experiment. The results are shown in figure 2.

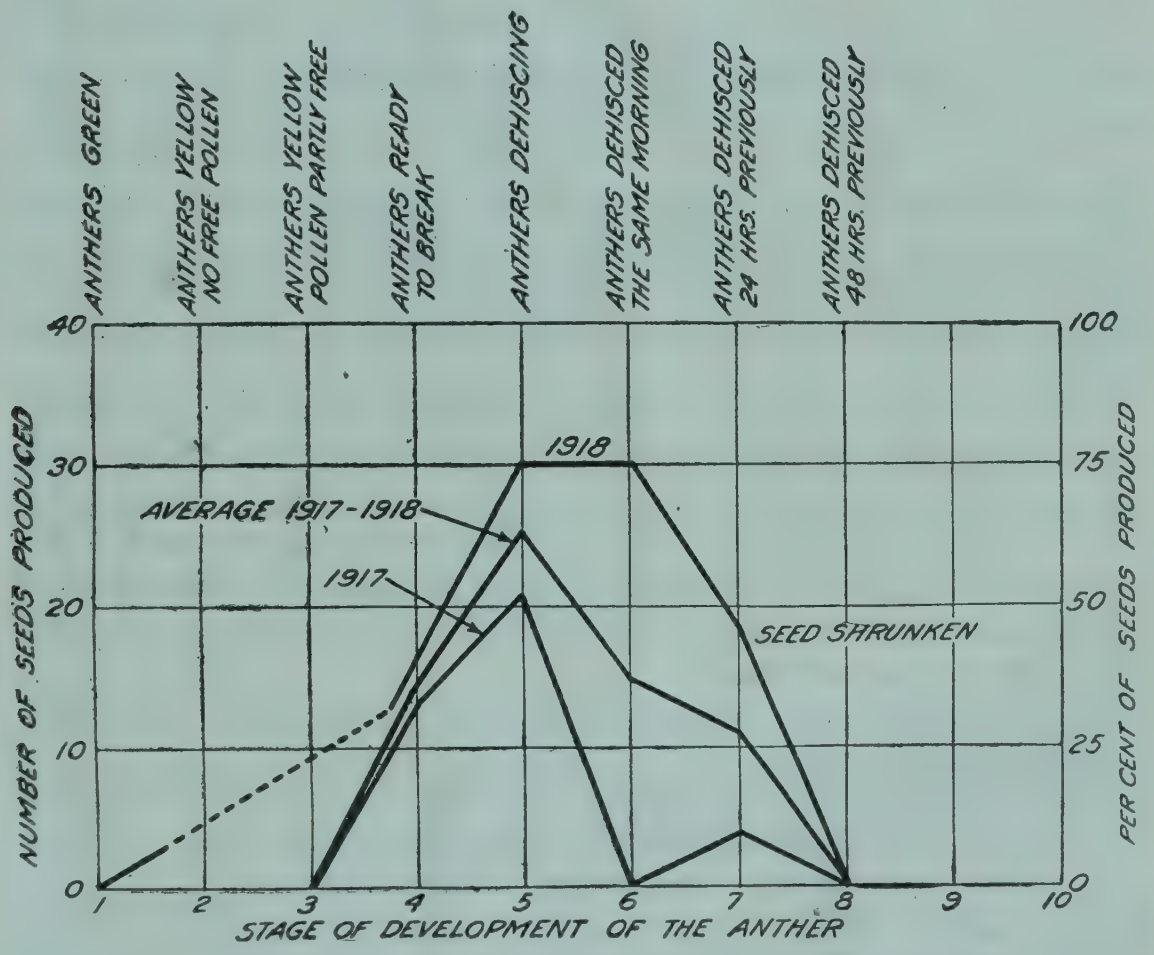

FIG. 2.—Number and percentage of seed produced by the use of pollen from anthers at various stages of development before and after dehiscence.

Anthers in eight different stages of development were used. In the first of these the anthers were green in color. In the second the anthers were yellow in color but did not yield dry pollen when broken. In the third the anthers were yellow in color, and pollen was secured from them only with difficulty. In the fourth stage the anthers were ready to break and yielded abundant dry pollen when ruptured. In the fifth stage the

anthers dehisced naturally. In the sixth stage the anthers had dehisced the same morning the filaments were extended. The base of the anther still contained considerable pollen, which was freed readily. The seventh stage was represented by anthers which had dehisced 24 hours previously. In this and in the eighth stage, in which the anthers had dehisced 48 hours previously, only a few still contained pollen. Only the best-appearing of this was used.

The period in which the pollen was found to be viable was very short. The best pollen was secured from anthers just breaking. Pollen from anthers ready to break gave a slightly lower percentage of successful pollinations. In 1918 the anthers used at this stage were slightly less mature than those used in 1917. Stamens with green anthers were used in both 1917 and 1918, but the two following stages were omitted in 1918. No seed was secured in either year from the use of immature pollen. These results are contrary to a widespread impression that detached anthers will complete the process of development, ripening, and fertilization when placed in a flower. By immature is not meant rudimentary pollen but pollen from anthers which, if left undisturbed, would not break for several hours. Pollen frequently can be dusted from such anthers by forcible rupturing of the walls. It will be recalled that this pollen swelled in water but did not burst. No artificial germination of such pollen was obtained.

The pollen remained highly viable in the field during only a few hours. Pollen taken from the anthers two or three hours before natural dehiscence effected fertilization in about 30 per cent of the attempts. Pollen taken from anthers which were dehiscing resulted in over 60 per cent of successful pollinations, while pollen remaining in anthers which had dehisced two or three hours previously effected fertilization in less than 40 per cent of the flowers pollinated.

In both years some fertilization resulted from pollen which had remained viable 24 hours after dehiscence. In one instance in 1918, not here reported, seed was secured from pollen of anthers which may have dehisced 48 hours previously. The age of the pollen in those classes after dehiscence is not certain. The stamens were left in the flowers, and the pollen remaining in the base of the anther was used. The age of the flowers was reckoned by means of observations on adjacent flowers and by other observations which have enabled the authors to determine the stage of development to within close limits.

In 1918 shrunken seed followed the use of pollen taken from anthers 24 hours after dehiscence. The numbers secured were not sufficient to indicate that the poor development was due to poor pollen.

It is the opinion of the authors that the success obtained with overripe pollen was due to the especially favorable conditions that prevail in Idaho. It is improbable, to the verge of certainty, that pollen could have remained viable in ruptured anthers 24 hours after dehiscence in a

humid climate. It would surely burst at night, when the condensation of moisture is heavy.

RETENTION OF VIABILITY IN THE LABORATORY

In the laboratory the attempts to keep barley pollen in such a way as to retain its viability met with no better success than in the field. Methods of keeping pollen of a few species of plants have been known for many years. Recently Pfundt (*9*) has been able to keep the pollen of many plants by regulating the amount of moisture in the air by means of solutions of dilute sulphuric acid of different water tensions. Swingle (*10*) and others have succeeded in keeping pollen by partial or complete drying in a vacuum. The laboratory attempts reported here were of three classes:

(1) Pollen left in the free air at 18° C. for different periods of time

(2) Pollen kept over sulphuric acid of different strengths.

(3) Pollen partially or completely dried in vacua at different temperatures.

In all cases the viability of the pollen was ascertained not only by artificial methods of germination but also by testing the germinative power directly upon the barley stigma. It was found advantageous to follow the growth by means of reagents, although the preliminary stages are characteristic and readily seen. Pollen brought in contact with the stigma attaches itself by means of some adhesive substance present, not only on the entire surface of the stigma hairs but also on the pollen grain itself. Normal viable pollen shows a distinct swelling, evidenced by different bulgings of the surface of the grain, which gradually disappear. The swelling is followed by a protrusion of the tube. This protrusion is very short, the tube bending immediately upon the grain, exhibiting a distinct contact tropism. The rapidly growing tube soon enters and disappears within the stigma hairs. The whole process of germination lasts from two to four minutes. To follow and differentiate the tube, the preparations were stained with a dilute solution of methyl-green acetic acid. This stains the pollen grain bluish and the contents of the tube light green. If a dilute solution of Congo red is applied subsequently, the tube is colored red, while the grain, which is unaffected, remains blue. Prolonged staining with Congo red will color the grain violet, the stain being so stable that it persists even after washing with a 5 per cent solution of sodium carbonate. Pollen in various stages of germination is shown in Plate 60. In Plate 60, the tube is still attached to the stigma, but the pollen grain has been loosened so as to make it more easily distinguished.

POLLEN LEFT IN FREE AIR

Pollen left exposed to the air for only two minutes loses so much moisture that it becomes shrunken. Shrunken pollen, however, does not mean necessarily a nonviable pollen. Such pollen may germinate,

provided its moisture content does not reach a dangerously low level. Normal pollen is shown in Plate 61, A. The same pollen exposed for 2 minutes to the air is shown in Plate 61, B. Some of this pollen may germinate. As previously stated, however, when the pollen was exposed to the air for 10 minutes, it had lost its germinative properties completely. This shrunken pollen, when applied to the stigma, affected the stigma hairs noticeably. The pollen grains took up moisture, attaining the round appearance of normal pollen; but the stigma hair cells lost their turgor and became vacuolized. It was thought that this effect upon the stigma hair cells might be overcome by swelling the pollen previous to its application to the stigma, but pollen so treated did not germinate.

Since the viability was lost so quickly in free air, it was obvious that more elaborate experiments of this nature were useless. While not strictly in free air, pollen remained viable 24 hours in a cool, dark room, when inclosed in a loose Van Tieghem cell with a piece of pea leaf as a moisture-giving medium.

POLLEN KEPT OVER SULPHURIC ACID

The trials in free air indicated that the regulation of the moisture content of the air was perhaps the most important factor in preserving pollen. Pollen was kept over sulphuric acid of different concentration as follows:

15.14 per cent with a 90 per cent moisture saturation;

37.69 per cent with a 60 per cent moisture saturation;

54.00 per cent with a 30 per cent moisture saturation.

Germination was later secured only from the 90 per cent moisture concentration. The experiment was not satisfactory in that the control was not accurate. This was especially true of the temperature. From all the observations made, it is the opinion of the authors that in the presence of a considerable range of humidity, pollen must remain viable for some time at a temperature of about 10° C.

POLLEN KEPT IN VACUA

The consequence of exposing pollen to the air were so obvious that experiments in vacua seemed foredoomed to failure; but citrus pollen had been preserved so successfully in this way that the same method was employed with barley pollen.

The pollen was put in gelatin capsules, care being taken not to have them completely closed. The capsules were placed in test tubes, which were subsequently evacuated, the vacuum being regulated to the millimeter pressure wanted. In no instance did barley pollen remain viable. The pollen was invariably shrunken and would not germinate under any conditions.

DISCUSSION OF RESULTS

The outstanding feature of all experiments with barley pollen is the extreme delicacy of the water adjustment of the pollen grain. Exposed to dry air for two or three minutes, the walls collapse through the loss of moisture. Exposed in a saturated atmosphere, the same cell imbibes water so fast that it bursts in even less time.

The water content of barley pollen is not known to the writers, nor has the percentage or the rate of loss of moisture been ascertained. It is known, however, that pollen of *Zea mays*, left under natural atmospheric conditions, loses from 40 to 50 per cent of moisture in a short time. Barley pollen may lose even higher percentages in the same length of time, if its sensibility to water is used as a criterion.

The imbibition of water occurs irrespective of the age of the pollen and probably occurs in nonviable as readily as in viable pollen. In aqueous solutions, immature pollen took up water and increased in size. Mature pollen under the same circumstances took up water but did not increase in size. The expansion of the contents resulted in bursting or in the protrusion through the pore of the intine and part of the cell contents. It seems logical to conclude that the cell wall of immature pollen becomes hardened by full maturity so that it resists the pressure of expansion.

When mature pollen is applied to the stigma the cell wall becomes cemented to the stigma hair, effecting a union sufficiently strong to furnish an anchorage from which forcible entry can be made into the stigma hairs.

The actual germination or growth is a complex process attributable to the specific physicochemical properties of the protoplasm and its properties of imbibition. The imbibition pressure is a deciding factor in primary growth. McDougal (*6*) believes this is the case in growing cells in other tissues. Osmotic pressure apparently is a negligible quantity at first, but it is of high importance in the later stages of growth.

The protoplasm is considered an emulsion colloid of a diphasic character and reversible in reaction. It possesses highly hydrophilic properties, and because of its high viscosity possesses also a high diffusibility for water. The behavior of barley pollen under the diverse physical conditions of moisture and temperature leads to the assumption that the reversible system from sol to gel is not pronounced, minute changes of moisture being able to destroy completely the balance of the system in one direction. It is true that germination may be accomplished after the cell walls have begun to collapse, if the loss of water has not reached the point where irreversible processes may take place. The loss of viability of barley pollen left for several minutes in free air may also be explained by the formation of synthetic products dehydrolitic in character, which are toxic and inhibit germination.

What part is played by the lipoid content of the protoplasm and its spatial relation to the disperse medium in those different colloidal processes we can only surmise, since the chemistry or even the quantitative analysis of pollen is not well known. Lloyd (*4*, *5*) infers that there may be such a relation.

The extreme sensitiveness of the pollen grain of barley to external conditions might be expected to lead to considerable sterility in the field. In most varieties, however, there is very little sterility. This, doubtless, is due to an equally delicate adjustment of the dehiscence of the anther. Development of the anthers seems to be entirely checked on cold, wet days. There may be very little fertilization for two or three days in succession during a period of adverse weather conditions. The development or at least the dehiscence of the anthers is retarded at night as well. In the morning, especially if the morning be clear, there is a very rapid evaporation of the dew and other condensed moisture from the surface of the plant. A little later the anthers begin to dehisce. This rupturing of the anther apparently occurs when its temperature rises and its surface begins to dry. This seems to be the time most favorable for the growth of the pollen as well. Conditions unfavorable for the growth of pollen are unfavorable for dehiscence.

The large number of anthers breaking in the early part of the forenoon indicates that those ripening since the preceding day had delayed dehiscence until favorable conditions obtained. After the very active period of the forenoon little fertilization occurs until midafternoon. It would seem that most of the anthers near ripeness had burst during the favorable period of the morning; and, except in scattering florets, renewed activity had to await the maturation of the next group of anthers. It is also possible that the extreme temperatures of midday may retard dehiscence.

There are a few varieties which exhibit infertility in the field. Primus, perhaps, is the most conspicuous of these. In Minnesota, in some years, finely developed spikes may produce but three or four kernels. In the West this variety is much more fertile. The cause of its infertility in Minnesota has not been ascertained by the writers, since neither of them has been at the St. Paul Station at flowering time since the pollen experiments were developed. It may be that the anthers burst at inopportune times or that many stamens may remain undeveloped. The senior author found the latter condition to exist in the greenhouse in Washington.

The experiments in keeping barley pollen did not lend any encouragement in this phase of the study. In practice, two suggestions may be offered. Pollen can be kept several days by picking the spikes just before the pollen matures and putting them in an ice box. The low temperature will prevent further development for a time. When needed, they can be placed with the stems in a glass of water in a warm room,

and they then will complete their development. If it is apparent sometime in advance that the desired varieties are not going to flower at the same time, the spikes of the more advanced variety should be removed. This will cause a good development of the secondary culms, which may flower about the time of the later sort.

The methods developed for the germination of barley pollen appeared to offer advantages which might be used with other species of grasses. Large percentages of germination were obtained with wheat pollen under conditions identical with those used for germination of barley pollen, except that the pollen of wheat was slightly less sensitive to the moisture.

SUMMARY

The artificial germinination of pollen of grasses has long been known to be a difficult matter. In a study of the factors affecting viability of barley pollen, its relation to moisture was found to be very delicate. Slight drying caused collapse of the walls, and free moisture caused rapid swelling and bursting. The relation of temperature also is critical.

Experiments were conducted to determine viability in various solutions and in different moist chambers. The former resulted in failures; the latter, in success under delicately controlled conditions. A proper range of humidity must coincide with a certain range of temperature.

Extensive studies were made of fertilization under field conditions, using pollen in eight successive stages of development, from immature to that obtained two days after dehiscence. Sixty per cent of seed formation was obtained when ripe pollen was used.

The retention of viability by barley pollen when stored under various conditions was studied. Results were determined by artificial germination and by germination on the stigma. No satisfactory results were obtained.

Study of the conditions governing fertilization in nature shows that conditions unfavorable to fertilizations are also unfavorable to progress in the development of pollen and vice versa. In this way natural fertilization is assured.

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PLATE 60

A.—Normal pollen.

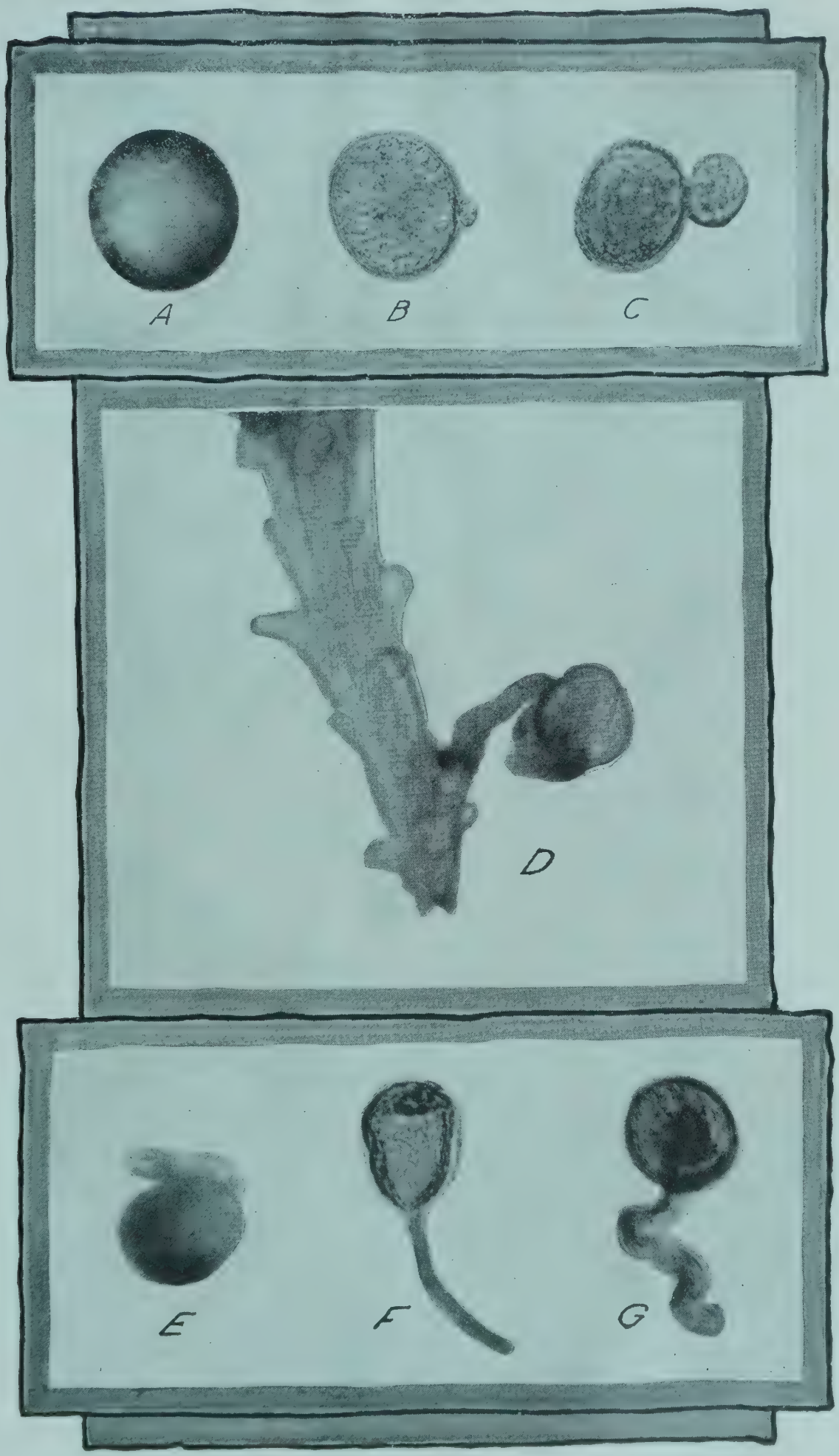
B.—Bulging of the intine through imbibition.

C.—The effect of free water when the intine is not ruptured.

D.—Pollen germinating on a stigma hair; the pollen grain loosened so as to show the tube.

E.—Germinating pollen.

F, G.—Pollen tube.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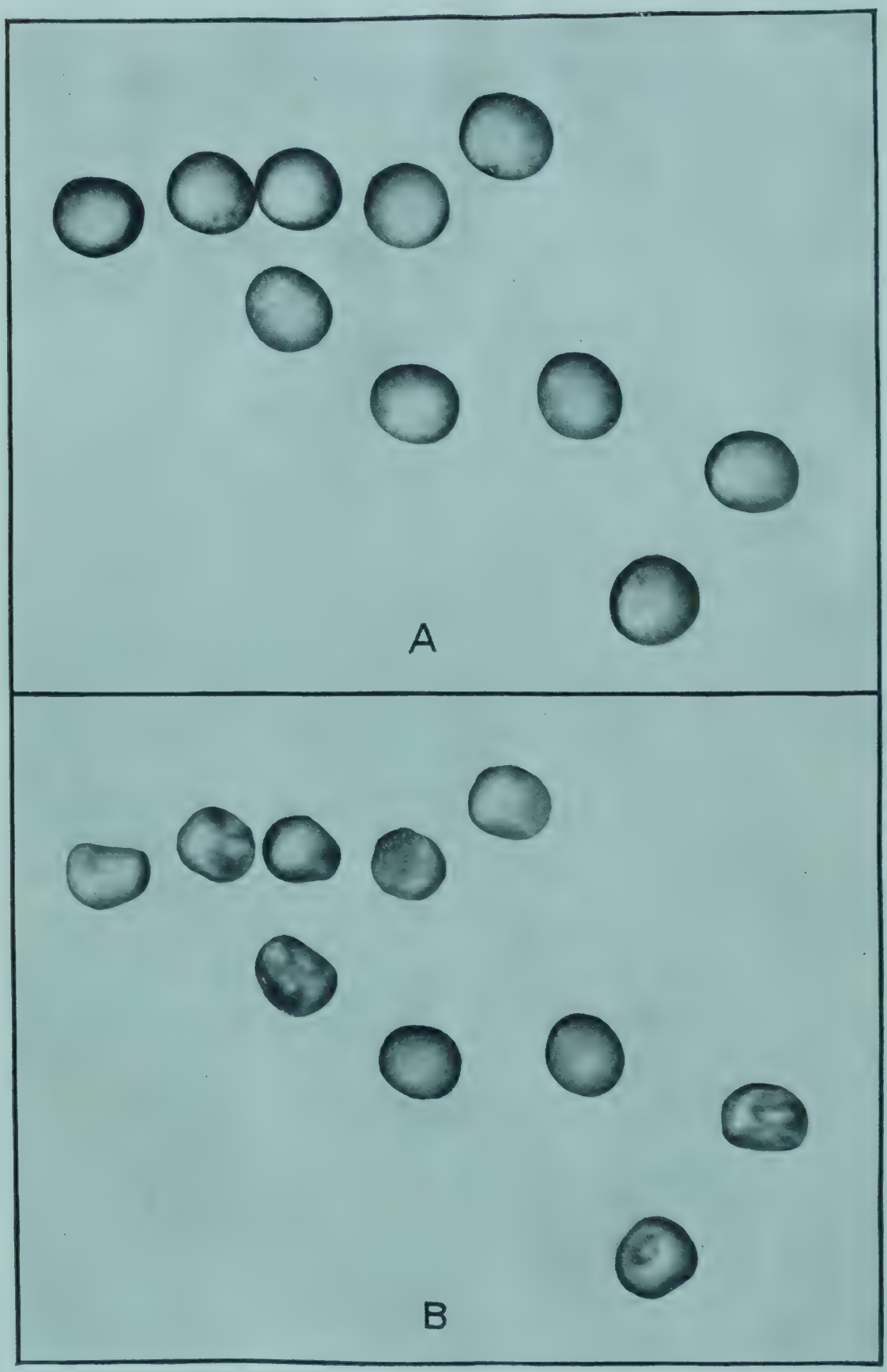
A
B

PLATE 61

A.—Normal pollen grains.
B.—Same pollen grains after an exposure of two minutes to dry air.

INVERTASE ACTIVITY OF MOLD SPORES AS AFFECTED BY CONCENTRATION AND AMOUNT OF INOCULUM

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Department of Bacteriology, Louisiana Sugar Experiment Station

It has previously been shown [2] that mold spores may release sufficient invertase to cause an appreciable inversion in 10 and 20 per cent sucrose solutions. This phenomenon was studied in connection with the deterioration of cane sugar by fungi [3], but in considering its bearing upon the latter problem it becomes imperative to establish the limits of concentration at which the invertase of mold spores is active. The work of O'Sullivan and Thompson [4] is responsible for the claim that invertase is not active in sucrose solutions above 40 per cent. The writers have been unable to find in the literature any contrary stand, although there exists some indirect evidence on this point. Since the film surrounding the sugar crystal capable of supporting biologic activity must vary in concentration from a supersaturated solution down to comparatively low concentrations (depending upon the amount and distribution of the moisture present), it was considered advisable to employ concentrations of 10, 20, 30, 40, 50, 60, and 70 per cent, at an incubation temperature of 48° to 50° C. for three days. The spore suspensions were prepared according to the method previously described.[2]

The experiment with blue aspergillus[5] is reported in Table I.

In Table I are presented the averages of closely agreeing triplicate determinations obtained with the spores of the blue aspergillus, at the rate of 144,000 spores per cubic centimeter for 10 to 40 per cent solutions of pure sucrose, and 130,000 spores per cubic centimeter for 50 to 70 per cent solutions. It will be noted that with an increase in concentration up to 60 per cent there is a proportional loss in percentage of sucrose and correponding increase in reducing sugars. Beyond this point the amount of inversion decreases. This brings out two important facts—namely, that the invertase in the spores of this mold is active in a sugar solution at the point of saturation, and furthermore that the maximum activity occurs between 50 and 60 per cent concentration for such an inoculation. It may be mentioned, parenthetically, that the polarization

[1] The authors are indebted to Lillian Kopeloff and the Station staff for many valuable suggestions.

[2] KOPELOFF, Nicholas, and KOPELOFF, Lillian. DO MOLD SPORES CONTAIN ENZYMS? *In* Jour. Agr. Research, v. 18, no. 4, p. 195–209. 1919.

[3] ——— ——— THE DETERIORATION OF CANE SUGAR BY FUNGI. La. Agr. Exp. Sta. Bul. 166, 72 p. 1919. Literature cited, p. 69–72.

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[5] Now identified as *Aspergillus sydowi* Bain.

of 10 to 40 per cent solutions was made directly (using a 100-mm. observation tube where necessary) while 26 gm. in 100 cc. were polarized in the 50 to 70 per cent solutions. Consequently, the conversion of polarization to percentage of sucrose in the 40 per cent solutions is a calculation based upon an assumed Brix and specific gravity.

TABLE I.—*Influence of concentration on invertase activity of spores of blue aspergillus*[a]

Concentration.	Polarization.	Loss in polarization.	Loss in sucrose.	Reducing sugars.	Gain in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 per cent, control	44.2	.....	.....	0.03	.....
10 per cent, with heated spores	44.0	0.2	.....	.03	.....
10 per cent, with spores	34.1	5.9	1.5	1.79	1.76
20 per cent, control	85.1	.....	.....	.07	.....
20 per cent, with spores	71.9	13.2	3.2	2.78	2.71
30 per cent, control	124.6	.....	.....	.12	.....
30 per cent, with spores	107.3	17.3	4.0	2.78	2.66
40 per cent, control	166.6	.....	.....	.14	.....
40 per cent, with spores	147.7	18.9	4.2	2.94	2.80
50 per cent, control	52.8	.....	.....	1.00	.....
50 per cent, with spores	47.4	5.4	5.4	4.41	3.41
60 per cent, control	57.7	.....	.....	1.71	.....
60 per cent, with spores	54.6	3.1	3.1	4.17	2.46
70 per cent, control	66.1	.....	4.6	2.13	.....
70 per cent, with spores	61.5	4.6	.....	4.54	2.41

[a] 144,000 spores per cubic centimeter were added to the 10 to 40 per cent solutions and 130,000 spores per cubic centimeter to the 50 to 70 per cent solutions.

The results of the experiment with the spores of *Penicillium expansum* are given in Table II.

TABLE II.—*Influence of concentration on invertase activity of spores of Penicillium expansum*[a]

Concentration.	Polarization.	Loss in polarization.	Loss in sucrose.	Reducing sugars.	Gain in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 per cent, control	40.0	.....	.....	0.03	.....
10 per cent, with heated spores	40.0	.....	.....	.03	.....
10 per cent, with spores	24.8	15.2	3.8	2.77	2.74
20 per cent, control	82.5	.....	.....	.17	.....
20 per cent, with spores	58.3	24.2	5.8	4.17	4.00
30 per cent, control	122.6	.....	.....	.21	.....
30 per cent, with spores	95.3	27.3	6.4	5.00	4.79
40 per cent, control	170.7	.....	.....	.23	.....
40 per cent, with spores	128.2	42.5	9.5	5.77	5.24
50 per cent, control	53.0	.....	.....	.97	.....
50 per cent, with spores	49.9	3.1	3.1	2.87	1.90
60 per cent, control	57.7	.....	.....	1.71	.....
60 per cent, with spores	57.6	.1	.1	2.63	.92
70 per cent, control	66.1	.....	.....	2.13	.....
70 per cent, with spores	64.5	1.6	1.6	2.73	.60

[a] 5,600,000 spores per cubic centimeter were applied to the 10 to 40 per cent solutions and 220,000 spores per cubic centimeter to the 50 to 70 per cent solutions.

It is to be regretted that the inoculations differed so widely, for there were 5,600,000 spores per cubic centimeter in the 10 to 40 per cent solutions and only 220,000 spores per cubic centimeter in the 50 to 70 per cent solutions. However, it is evident that with an increase in concentration up to 50 per cent there is a marked loss in sucrose accompanied by a corresponding increase in reducing sugars. Beyond this point the loss in sucrose is not so significant. The same is true of the gain in reducing sugars. One may be permitted the speculation that had the inoculation in the 50 to 70 per cent solutions been 25 times larger, the results would have corroborated those previously obtained with the blue aspergillus—namely, that the invertase released by the spores of *Penicillium expansum* was active in concentrations of sugar as high as the saturation point and attained a maximum between the 50 and 60 per cent concentrations.

In Table III are recorded the results obtained with *Aspergillus niger* in concentrations above 50 per cent sucrose, since we have previously indicated an increase in invertase activity of the spores of this organism with an increase in concentration.[1]

TABLE III.—*Influence of concentration on the invertase activity of spores of Aspergillus niger* [a]

Concentration.	Polarization.	Loss in polarization.	Loss in sucrose.	Reducing sugars.	Gain in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
50 per cent, control	52.0	.....	.....	1.16	.....
50 per cent, with heated spores	52.0	.....	.....	1.40	0.24
50 per cent, with spores	40.6	11.4	11.4	6.94	5.54
60 per cent, control	57.7	.....	.....	1.71	.....
60 per cent, with spores	48.1	9.6	9.6	6.67	4.96
70 per cent, control	66.1	.....	.....	2.13	.....
70 per cent, with spores	57.0	7.1	7.1	5.18	3.05

[a] 680,000 spores per cubic centimeter were used.

It will be seen from Table III that the percentages of sucrose lost and of reducing sugars gained show a maximum at 50 per cent concentration with *Aspergillus niger*, and that there is a diminished but significant activity up to the saturation point as with the blue aspergillus and *Aspergillus niger*.

While it is indeed difficult to draw any comparative conclusions concerning the invertase activity of the three molds under consideration, because of the differences in the number of spores used for inoculation, nevertheless it can safely be postulated that the spores of the blue aspergillus are relatively more active in inverting power than the other two molds employed. For it will be seen that with 130,000 spores of this

[1] KOPELOFF, Nicholas, and KOPELOFF, Lillian. THE DETERIORATION OF CANE SUGAR BY FUNGI. La. Agr. Exp. Sta. Bul. 166, 72 p. 1919. Literature cited, p. 69–72.

mold per cubic centimeter at the highest concentration employed (which was at the saturation point) there was a gain of 2.41 per cent in reducing sugars over the control, while with double the number of spores of *Penicillium expansum* there was only one-fourth that gain in reducing sugars. Five times as many spores of *Aspergillus niger* produced one-fourth greater increase in reducing sugars.

In general, then, it will be seen that the invertase activity of these mold spores is directly influenced by concentration in the manner described, and furthermore that it is likewise dependent on the number of spores employed. The second part of this paper, therefore, is concerned with the influence of numbers of mold spores upon their invertase activity in sugar solutions at the saturation point.

Since there has been occasion in another connection [1] to study the influence of the number of mold spores, somewhat the same procedure was employed in this experiment. The spore suspensions were prepared as described in an earlier paper,[2] and the proper dilutions made with sterile distilled water. The same conditions of incubation, etc., were again observed. The control flasks contained a mixture of spores of all dilutions heated to 100° C. for ½ hour. The largest number of spores of *Penicillium expansum* and *Aspergillus niger* was about 400,000 per cubic centimeter, and the largest number of blue aspergillus was about 80,000. The dilutions represented an arithmetic progression of one-half of the preceding quantity.

In Table IV will be found results obtained with varying quantities of inoculum in a sugar solution at the point of saturation.

Some striking differences are to be noted where the blue aspergillus was used. With a decrease in quantity of inoculum there is a proportional decrease in invertase activity until 5,000 spores are employed, at which point no significant inversion occurs. Consequently that number may be taken as the lowest limit essential to invertase activity in such a highly concentrated sugar solution.

On the other hand, *Penicillium expansum* is not nearly so effective, for it will be seen that while there is a decrease in invertase activity with a decrease in number of spores, nevertheless the minimum is reached between 110,000 and 55,000 per cubic centimeter, which is practically 10 times as great an amount as was required by the blue aspergillus. The lower limits of the invertase activity of *Penicillium expansum*, however, are very sharply defined. The results obtained with *Aspergillus niger* are practically identical qualitatively with those observed for *Penicillium expansum*, but vary in the quantitative relationships. To a certain degree this is probably accounted for by the fact that the latter mold had the larger inoculation.

[1] KOPELOFF, Nicholas. INOCULATION AND INCUBATION OF SOIL FUNGI. *In* Soil Sci., v. 1, no. 4, p. 381–403. 1916. Literature cited, p. 402–403.

[2] ——— and KOPELOFF, Lillian. DO MOLD SPORES CONTAIN ENZYMS? *In* Jour. Agr. Research, v. 18, no. 4, p. 195–209. 1919.

TABLE IV.—*The influence of numbers of spores on the invertase activity of mold spores in 70 per cent sugar solutions*

BLUE ASPERGILLUS

Number of spores per cubic centimeter of solution.	Polarization.	Loss in sucrose.	Reducing sugars.	Gain in reducing sugars.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Control	66. 5	.....	0. 97	.....
80,000	59. 7	6. 8	4. 55	3. 85
40,000	63. 1	3. 4	2. 78	1. 81
20,000	64. 7	1. 8	2. 18	1. 21
10,000	65. 0	.5	1. 50	. 53
5,000	65. 3	1. 0	1. 07	. 10

PENICILLIUM EXPANSUM

Control	66. 3	.....	1. 50	.....
440,000	61. 1	5. 2	3. 57	2. 07
220,000	63. 8	2. 5	2. 67	1. 17
110,000	64. 9	1. 4	1. 90	0. 40
55,000	65. 2	1. 1	1. 48	0
28,000	66. 1	. 2	1. 48	0

ASPERGILLUS NIGER

Control	66. 4	.....	0. 81	.....
400,000	64. 0	2. 4	1. 31	0. 50
200,000	65. 3	1. 1	1. 00	. 19
100,000	66. 5	.....	1. 07	. 26
50,000	66. 3	. 1	. 86	. 05
25,000	66. 3	. 1	. 88	. 07

While the limitations of the data herein presented are clearly recognized to the extent that it is known to be exceedingly hazardous to state the limits of enzymic activity in terms of such units, because of the variations which must exist between different strains of the same species, nevertheless the results are suggestive in their bearing upon the practical problem of sugar deterioration. A certain correlation to be emphasized is that the blue aspergillus, which was found to have the greatest capacity for deteriorating sugar (besides occurring with greatest frequency), has spores which appear to exhibit the most intense invertase activity in saturated sugar solutions. Since we have pointed out that sugar inoculated with spores of this mold deteriorated without the development of mycelia, the conclusion would appear to be substantiated that mold spores alone, if present in sufficient number, are capable of deteriorating sugar.

SUMMARY

(1) The invertase activity of the spores of blue aspergillus, *Aspergillus niger*, and *Penicillium expansum* is exhibited at concentrations of sugar varying from 10 to 70 per cent.

(2) The maximum invertase activity of those mold spores occurs between 50 and 60 per cent concentrations.

(3) An increase in the number of mold spores is responsible for increased invertase activity in a saturated sugar solution.

(4) The least number of spores of *Penicillium expansum* and *Aspergillus niger* required per cubic centimeter to produce inversion in saturated sugar solution is between 50,000 and 110,000. About 5,000 spores of blue aspergillus are needed to cause inversion.

(5) The evidence that mold spores alone are capable of deteriorating cane sugar is corroborated by the data herein presented.

BASAL GLUMEROT OF WHEAT

By Lucia McCulloch

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In the course of an examination in this laboratory of various collections of wheat of the crop of 1917 made for the study of "black chaff," a bacterial disease unlike "black chaff" or any other reported wheat disease was discovered; and this same disease was observed again several times in the collections of 1918.

This disease affects the leaf, head, and grain of wheat. On the heads the glumes show at the base a dull brownish black area. Sometimes this dark area extends over nearly the whole surface of the glume; but usually only the lower third, or less, is darkened (Pl. 62, A, B); and often no discoloration is visible on the exterior. Glumes that have a normal color on the outer surface may have the inner surface discolored. In practically all cases, dissection of the spikelet reveals more signs of disease on the inner surfaces than on the outer. Often a narrow dark line at the junction of the spikelet and the rachis is the only outward sign of the disease.

The grain inclosed by such diseased glumes shows varying degrees of undevelopment. The fact that grains are often well filled out would indicate that the disease sometimes appears late in the course of growth. In diseased grain the base, or germ end, varies in color from a scarcely noticeable brown to charcoal black (Pl. 62, C). In severe cases the surface texture as well as the color suggests charring. In the discolored areas bacteria are found in great abundance. Pure cultures have been secured from material collected in various States and in Canada.

This type of disease has been found in collections of wheat from New York, Michigan, Kansas, Missouri, Minnesota, North Dakota, South Dakota, Oklahoma, and Alberta, Canada.

The bacterium isolated from the infected glumes and grains is a medium-sized rod (1 to 2.7μ by 0.6μ), with rounded ends; in favorable media it forms long chains; it is actively motile by means of one to four polar or bipolar flagella (Pl. 63, F); and capsules are present in 6-day-old beef agar cultures (Ribbert's capsule stain).

No spores, zoogloeae, or involution forms have been observed.

The organism is Gram-negative and is not acid-fast.

Its staining reactions are rather feeble, but hot carbol fuchsin, anilin gentian violet, and saturated gentian violet gave good results. The stains must be washed out only with weak grades of alcohol (40 to 50 per cent), since strong alcohol takes them out too readily.

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Growth is good in the usual culture media, though the peptone-beef media are less favorable for growth and retention of vitality than potato, milk, or Uschinsky's solution.

CULTURAL CHARACTERS

AGAR POURED PLATES.—On peptone-beef agar the colonies become visible in about two days at room temperature and if well isolated reach a diameter of from 6 to 10 mm. in six days. The colonies are white or rather like boiled starch and translucent. After several days the color is greenish white. The bacteria in mass on a white background are yellowish green. The medium immediately surrounding the colonies becomes greenish, and this color eventually spreads over the whole plate. A single colony greens about one-fourth of an ordinary plate. The texture is at first soft and tender, and the colonies coalesce readily. If the plate is tipped, the colonies overflow the lower margin. Later the growth is thicker but not viscid. The colonies are circular, margins entire and definite, surface shining and smooth, with age occasionally slightly contoured, finally drying to a whitish film as transparent as the dry agar medium. A characteristic interior structure is the presence of numerous, tiny striae concentrically arranged. Sometimes these are coarser, more "fish-scale" in character. Only rarely have colonies occurred with "fish-scale" marks predominating (see Pl. 63, B). More often the "fish-scale" marks have been observed at the margin of colonies. This coarse marking rapidly changes into the finer lines termed "striae." The colonies which show these "fish-scale" markings are extremely thin and transparent. The striations usually disappear after six or seven days, though they have been observed in some cases in colonies 2 weeks old (Pl. 63, C). Oblique light is necessary for examination of these internal markings. In reflected or direct transmitted light, colonies often show a marginal band more opaque than the centers (Pl. 63, D). Buried colonies are dense and opaque and more or less irregular in shape.

The usual type of colony is best illustrated by Plate 63, A, E, shown under oblique lighting. Slight diversities in colony character seem to be caused by various factors such as moisture content of medium, degree of acidity or alkalinity of medium, temperature, length of time in artificial media, etc.

WHEY AGAR.—Formula: 1,000 cc. whey, 300 cc. water, 15 gm. agar flour, 15 gm. peptone, 15 gm. cane sugar, 3 gm. gelatin. Colonies smaller but thicker than on beef agar. At first almost hemispherical, translucent, and with pearly luster. The smooth surface becomes slightly contoured, and after four weeks the colony looks like a miniature rugged mountain. Buried colonies are translucent and larger than in beef agar, and the green stain is less conspicuous.

POTATO AGAR PLUS 1 PER CENT DEXTROSE.—The organism grows well on this medium, forming thick, almost opaque, greyish white colonies.

Striae, often coarse, sometimes even "fish-scale" in character, may be seen in the margins. A halo forms about the colonies. These halos are not cleared areas, but rather a whitish clouding fading gradually into the unchanged medium of the plate. In crowded plates this clouding so quickly coalesces that no halos are observed. After five or six days the colonies have a zoned appearance, the centers and borders being rather white, separated by a greyish zone. The internal marks are finer and more like those seen in beef agar.

AGAR STABS.—Surface growth only moderate, white, moist, and shining. Slight mesenteric growth in the upper part of the stab, but this does not persist. Old cultures show only surface growth. The whole medium becomes pale yellowish green ("Bright Chalcedony Yellow").[1]

AGAR STREAKS.—The growth is white, thin, transparent. The medium greens in 24 hours. The surface is very slightly contoured. The internal markings—striae—show along the margins for several days. On the upper part of the slant the bacteria are in a very thin layer, becoming thicker toward the lower part and with white sediment in the condensation water in the V.

GELATIN PLATES.—(+10 peptone-beef gelatin). In 48 hours at 20° C. the well-isolated colonies are 1½ mm. in diameter. The liquefied pits are shallow, saucer-shaped depressions, circular and with definite, entire margins. The liquid gelatin is slightly clouded, and the bacteria are mostly in a tiny white mass at the center of the depression. In 4 days well-isolated colonies are from 6 to 8 mm. in diameter. Thickly sown plates are entirely liquefied in 48 hours at 20°, with the colonies as tiny white masses floating in the slightly cloudy and slightly greened liquid.

GELATIN STABS.—Cultures kept at 17° to 18° C. have small liquefied pits on the surface in two days. The stab does not develop more than a trace of growth. The liquefaction becomes stratiform and proceeds slowly, being complete only after from five to seven weeks. At a temperature of 20° the liquefaction is more rapid, and the stab develops into a wide pocket containing numerous small white masses. At both temperatures the gelatin is greened.

BEEF-PEPTONE BOUILLON.—At room temperatures (20° to 27° C.) a slight clouding occurs at the surface in from 6 to 7 hours. The growth increases, always being best at the surface, forming clouds and pseudo-zoogloeae. In some cases a delicate pellicle forms. Rims are often present, thin, white, easily disintegrating. Growth is never very heavy in +10 to +14 beef bouillon. The slight sediment is white, fine-grained to flocculent, and is readily dissolved into clouds on shaking. Medium yellowish green ("Chalcedony Yellow" or "Chartreuse Yellow").

BEEF BOUILLON OVER CHLOROFORM.—Growth unrestrained.

[1] The colors mentioned in this paper are given according to Ridgway (RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE, 43, p., 53 col. pl. Washington, D. C., 1912.)

NEUTRAL PEPTONE-BEEF BOUILLON.—Growth good, better than in + 10 beef bouillon. A pellicle forms. Medium greened.

LOEFFLER'S SOLIDIFIED BLOOD SERUM.—Only moderate growth and a very slow liquefaction with some strains of the organism. With one strain (No. 285) evidently no liquefaction.

POTATO CYLINDERS.—Growth at first dull, yellowish white, changing to a dirty, greenish brown ("Buffy Citrine"). The growth is moist and shining but not thick. The moister parts of the potato seem most favorable for growth. The diastatic action on the starch is moderate.

ACTION ON STARCH.—Potato cylinders on which the organism had grown for three weeks or more showed a moderate change in the starch. Tested with iodin the cultures gave reddish brown to purple reactions; the controls gave pure deep blue. This test was performed by crushing the cylinders and adding 10 cc. of water and 5 cc. saturated iodin in 40 per cent alcohol.

Peptone-beef agar plus 1 per cent potato starch was poured into Petri plates. When firm, the surface was inoculated by drawing a needle bearing bacteria across the center of the plate. Ten days later when the streaks were from 3 to 7 mm. wide the surface of the plates was flooded with iodin (saturated solution in 40 per cent alcohol). Most of the plate became intensely dark blue, except along the streak of bacterial growth where there appeared a clear area, or halo, from 2 to 6 mm. wide.

Under the microscope abundant starch particles could be seen in the agar, except in the halos where only a few scraps remained. A field in the halo gave by count only three tiny particles, while 10 cm. out in the blue agar a field of the same size gave over 200 large starch grains and many more small particles.

Plain peptone agar plus 1 per cent potato starch gave scantier growth to the bacteria, and the test with iodin showed no trace of halos.

Peptone-beef bouillon + 10 with 1 per cent potato starch.—Organisms were grown in this medium for 12 days. Then 2 cc. of iodin (saturated solution in 40 per cent alcohol) were added to the tubes. A reddish brown color developed, quickly and entirely fading out, while controls became and remained deep blue. Some of the same lot of cultures were tested with Fehling's solution. Reactions of cultures varied from orange to reddish purple; the controls were blue. After the cultures had settled for some hours a very slight reddish precipitate could be discerned in some of the tested tubes.

Uschinsky's solution plus starch.—A thin starch paste was added to Uschinsky's solution (about 20 cc. of starch paste to 100 cc. of the Uschinsky). Cultures grown in this for three and five weeks gave, when tested with iodin, brownish purple reactions. The controls were deep blue.

MILK.—Milk clears without coagulation. A watery band, rather greenish in color, begins at the surface and extends downward until the

whole tube is clear and translucent. The color is greenish yellow. With some strains this action is complete in five days, in others not until the twelfth day. Thin, white rims are present, no pellicles. The sediment is white, flocculent to curdy. In two months the color is light brown (between "Pinkish Buff" and "Cinnamon Buff") and the medium still translucent. No crystals are present.

LITMUS MILK.—In 24 hours the whole tube is slightly blued, and the surface shows a watery band of dark blue. This dark blue band deepens until in from 5 to 12 days the whole tube is very dark blue. In undisturbed cultures there may be seen three or more distinct bands of color, the darkest on the surface. In reflected light the color is almost black. There is no reduction. No further change was observed in two months. No crystals formed. In five different tests in litmus milk the results were uniformly as stated above. In a sixth test in a medium containing a smaller amount than usual of litmus, two strains (471 and 478) showed a trace of reduction on the tenth day. This did not increase, and on the fifteenth day the blue color was reestablished.

LITMUS AGARS WITH SUGARS.—On litmus-dextrose, litmus-saccharose, and litmus-galactose agar the medium was reddened within 24 hours. The acid action gradually increased for about 10 days then decreased, until after 5 weeks there remained almost no red color. One strain retained some red color in the dextrose and in the galactose media.

Litmus-lactose agar and litmus-glycerin agar never developed any red color. After five weeks the medium was a greenish blue, showing some change in the litmus.

Growth in these media was only moderate.

USCHINSKY'S SOLUTION.—A moderate to heavy growth is produced in this medium. Growth is best at the surface in the form of clouding, pseudozoogloeae, and a delicate pellicle. The medium becomes pale, apple green in color ("Veronese Green").

FERMI'S SOLUTION.—This gives an abundant growth. A delicate pellicle forms with clouding and pseudozoogloeae below. The color is pale bluish green ("Pale Veronese Green"). The pellicle becomes thicker and somewhat viscid. In two weeks it is about 2 mm. thick and difficult to break up.

COHN'S SOLUTION.—The organism does not grow in this medium.

TOLERATION OF SODIUM CHLORID.—Peptone-beef bouillons +13 containing 2, 3, 4, and 5 per cent of sodium chlorid were inoculated from young agar cultures. Growth occurred in all but was rather scanty in the 5 per cent. The experiment was repeated using 4, 5, and 6 per cent of sodium chlorid in +13 beef bouillon. Again growth was scanty in the 5 per cent. No growth occurred in the 6 per cent.

FERMENTATION TUBES.—The medium used was 2 per cent Witte's peptone and 1 per cent, respectively, of each of the following: dextrose, lactose, saccharose, maltose, mannit, glycerin, and levulose. The open

end of the tubes clouded in from 2 to 3 days, and in two weeks the growth was moderate to heavy. There was a very faint clouding in the closed ends with mannit, saccharose, and levulose. In the dextrose tubes the closed end seemed to be cloudy at first, but at the end of two weeks these were entirely like the controls. No gas formed in the closed arm in any media. When tested with litmus paper on the twentieth day the dextrose cultures gave an acid reaction, glycerin cultures were neutral, and the others—mannit, saccharose, maltose, lactose, and levulose—were alkaline. The control tubes were acid.

NITRATES ARE NOT REDUCED.—Growth in the nitrate-beef bouillon is good, and a pellicle forms. Cultures were tested when 10, 17, and 24 days old. Starch water, potassium-iodid solution, and sulphuric acid were used in testing for nitrite. No change of color occurred in any of the cultures—that is, no nitrite is formed.

HYDROGEN SULPHID is produced in small quantities as determined by the slight browning of lead-acetate paper suspended over cultures growing in beef bouillon, beef agar, and in milk.

INDOL is produced in small but definite amounts in the following medium: 1 per cent peptone, 0.5 per cent disodium phosphate, and 0.1 per cent magnesium sulphate in distilled water. Cultures in 1 and 2 per cent peptone (Witte's), Dunham's solution, and Uschinsky formed no demonstrable amounts of indol.

AMMONIA production is feeble. Beef bouillon cultures were tested with Nessler's solution. Strips of filter paper moistened in the solution were suspended over the cultures, which were then heated. A brownish color developed on the paper. A second test was made, using filter paper colored with haematoxylin. This changed from a reddish color to purple in the heated culture tubes.

TOLERATION OF ACIDS.—The toleration of acids was tested in +10 peptone-beef bouillon to which were added different percentages of citric, malic, tartaric, and hydrochloric acids. Sufficient acid was added to the bouillon to make 0.05, 0.10, 0.15, and 0.20 per cent solutions. One cc. of 0.5, 1, 1.5, and 2 per cent acid solutions, respectively, were added to 9 cc. of the +10 bouillon. The acid was added under sterile conditions and the solutions used four days later without heating. The organisms grew well in the 0.05 and the 0.10 per cent solutions of citric, malic, and tartaric acids. Growth was good within 24 hours. Heavy pellicles formed and the media became green. One strain (No. 471) grew in the 0.15 per cent solutions of citric, malic, and tartaric acids. All strains grew in the 0.15 per cent solution of hydrochloric acid. None grew in a 0.15 per cent solution of oxalic acid. No growth was obtained in any of the 0.20 per cent solutions with any of the strains of the organism.

TEMPERATURE RELATIONS.—In +10 peptone-beef bouillon the optimum temperature is 25° to 28° C. The maximum is between 36° and 37°,

and the minimum is below 2°. The thermal death point is between 48° and 49°.

DESICCATION.—Bacteria from 24-hour beef bouillon cultures, spread on sterile cover glasses and kept in the dark, were nearly all dead inside of 24 hours. A few grew after 7 days' drying.

Bacteria from 7-day-old agar cultures showed more resistance and were alive on the ninth day after being put on covers. A few were alive on the twelfth day, and one culture was secured after 26 days. The organism has been isolated from dry wheat kernels kept at room temperature for 17 months.

OPTIMUM REACTION IN BEEF BOUILLON.—The organism grows well in peptonized beef bouillons titrating from 0 to −14 on the alkali side and to +30 on the acid side. Beyond these limits the growth was feeble or lacking. Sodium hydrate was used for the alkali and hydrochloric for the acid. These were inoculated rather heavily from 2-day-old beef bouillon cultures.

EFFECT OF SUNLIGHT.—Five minutes' exposure to sunlight, on ice, killed about 50 per cent of the organisms in agar plates. Ten minutes' exposure caused a reduction of from 95 to 100 per cent.

EFFECT OF FREEZING.—Several tests were made. (1) Freshly inoculated beef bouillon cultures were frozen for two hours. The reduction of the organism was 70 to 75 per cent. (2) Five hours' freezing caused a reduction of from 84 to 100 per cent. (3) Forty-four hours' freezing killed 80 to 100 per cent.

NAME OF ORGANISM

This organism appears to be an undescribed form, and because of the brown to black discolorations which it causes at the base of the wheat kernels and glumes the name *Bacterium atrofaciens*, n. sp., is suggested.

TECHNICAL DESCRIPTION

Bacterium atrofaciens, n. sp.[1]

Rods, cylindrical, rounded at ends. Individual rods 1 to 2.7μ by 0.6μ. In liquid media, moderately long filaments (3 to 30 bacteria). Motile by 1 to 4 polar flagella; aerobic, no spores; capsules in beef agar cultures.

Surface colonies in peptone-beef agar plates are round, smooth, shining, and with inconspicuous internal striations, somewhat irregularly concentric in arrangement, white becoming greenish, the surrounding medium also becoming greenish.

[1] *Bacterium atrofaciens*, sp. nov.

Baculis asporis, aerobiis. Capsulas in culturis agar-agar habentibus. Baculis singularibus 1–2.7μ×0.6μ. 1–4 flagellis polaribus mobilibus. In culturis liquidis 3–30 baculis in filamentis conjunctis. Coloniis albis, rotundis, nitentibus, et in agar-agar cum striis inconspicuis internis, aliquid irregulariter concentricis; Culturae et coloniae virescentes.

Gelatinam liquefacit. Nitrum non redigitur. Lac sterile alcalinum fit, non coagulatur. Saccharum sacchari, saccharum uvae, et saccharum galacti acidum fit; gas non fit. Baculi methodo Gram non colorantur.

Habitat in foliis, glumis, et granis *Tritici vulgaris*. Maculas fuscas in basi glumarum granorumque facit.

Gelatin is liquified; milk becomes alkaline and translucent but does not coagulate; nitrates not reduced; acids produced from saccharose, dextrose, and galactose. No gas produced. Gram-negative. Group number 221.2322123.

Pathogenic to *Triticum vulgare*, causing especially discoloration and lesions at the base of glumes and kernels.

INOCULATIONS

Inoculations on young wheat plants have given numerous leaf infections (Pl. 62, D). Fewer mature plants have been available for inoculation of heads, and weather conditions were rather unfavorable; but enough infections were secured to determine the positive pathogenicity of the organism for glumes (Pl. 62, E, F) and kernels.

For the leaf inoculations bacteria from agar subcultures were mixed with sterile water and sprayed on the plants with an atomizer. The infections are stomatal. On the second day after the inoculation, dark, water-soaked spots appear. These are small and well distributed over the leaf surface. Two days later the spots are pale yellow. They enlarge and also elongate slightly. Later the color is light brown and the tissues are dry.

Sometimes bacteria were added directly to drops of condensation water on the leaf tips. In these inoculations the whole tip of the leaf became yellowish brown and shriveled. If examined at the end of five or six days the bacteria are abundant in the tissues. From the leaf infections (more than 50 series of successful inoculations) the organism was reisolated 22 times and often used again, producing typical infections.

For inoculating the heads of wheat, bacteria from agar subcultures were diluted in sterile water and by means of a camel's hair brush were spread over the glumes and between the spikelets of young heads just emerging from the flag leaf. On some glumes a few slight wounds were made with a fine needle.

The infections (all done in Washington, D. C.) were slower in showing on the heads than on the leaves. Glume discolorations were noted first on the fourth day after inoculation. The spots enlarged slowly and never became so noticeable as those on naturally infected heads. Examination after collection showed that the bacteria had penetrated to and into the kernels, some of which showed the characteristic blackening at the germ end and great abundance of bacteria in the spaces about the radicle. The inner surface of the glumes also showed more discoloration than was visible from the exterior. (See Pl. 62, F, *a*.)

The characteristic organism was reisolated from both the glumes and the kernels of the inoculated heads and used again in a second series of inoculations on heads and on leaves of seedling plants. The disease was reproduced in both series, and the organisms were again isolated from each

SUMMARY

A bacterial disease of wheat caused by a hitherto undescribed organism has been found on heads of wheat collected in various and widely separated localities in the United States and Canada.

The most noticeable external character of the disease is the brown to black discoloration on the lower part of the glumes and of the adjacent rachis. The grains inclosed by the diseased glumes have bacteria in the tissues at the germ end. In advanced cases this end of the grain is black and charred in appearance.

The discolored tissues swarm with bacteria. These have been isolated not only from freshly collected material but also from grain kept in the laboratory for 17 months.

The parasite is a white, polar-flagellated rod, producing a green fluorescence in the ordinary culture media.

The group number is 221.2322123.

Bacterium atrofaciens, n. sp., is suggested as the name.

PLATE 62

Basal glumerot of wheat:

A.—Heads showing diseased glumes. Collected June, 1917. Kansas. Photographed May, 1918. ×1½.

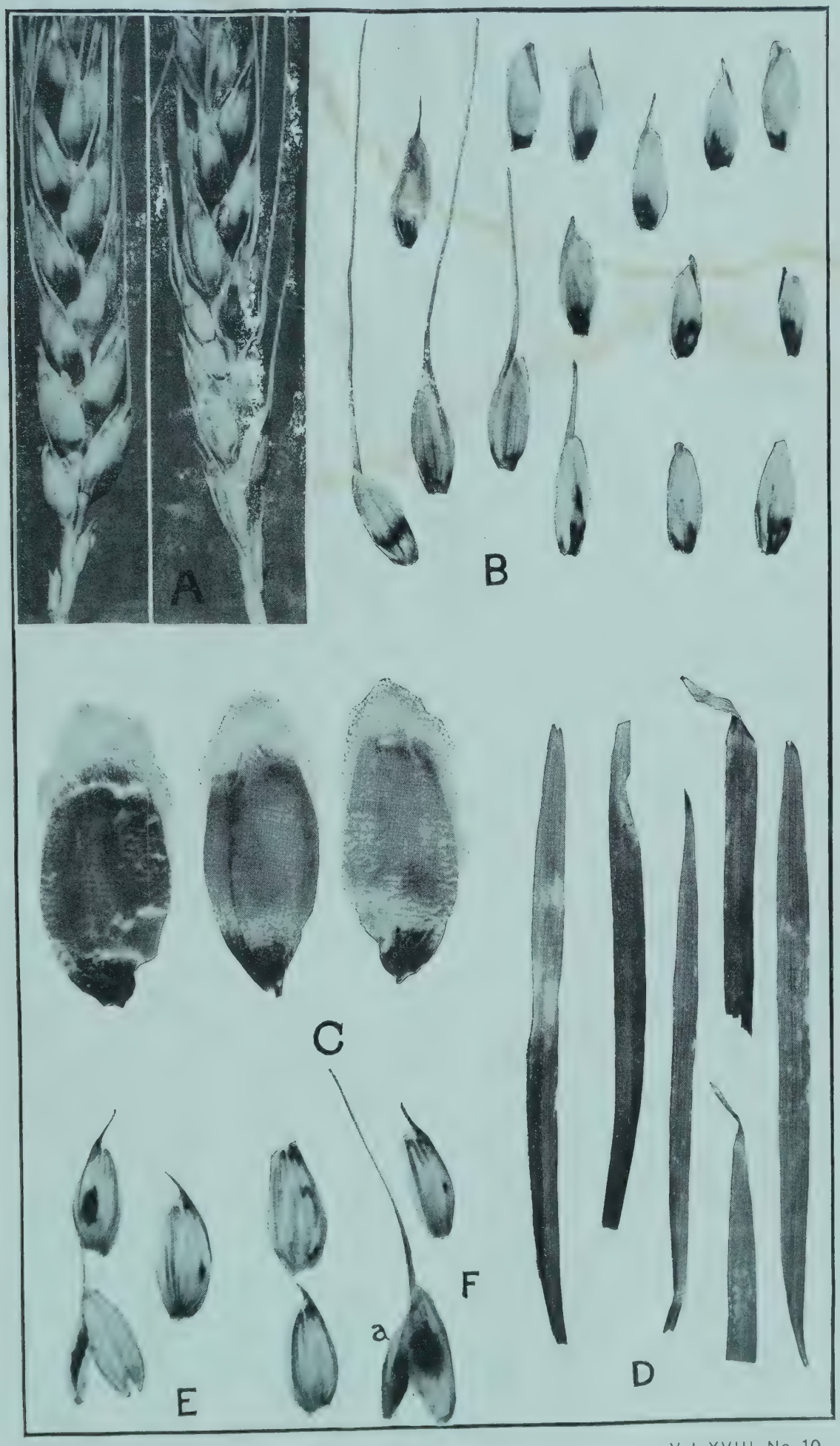
B.—Glumes diseased at base. Collected in New York and Alberta, Canada. ×1½.

C.—Grains black and full of bacteria at base. From New York and Canada. ×6.

D.—Young wheat leaves five days after inoculation with *Bacterium atrofaciens*.

E.—Glumes inoculated with strain 478. May, 1918.

F.—Glumes inoculated with strain 399. *a*, Inner face. May, 1918.


A
B
C
D
E
F
a

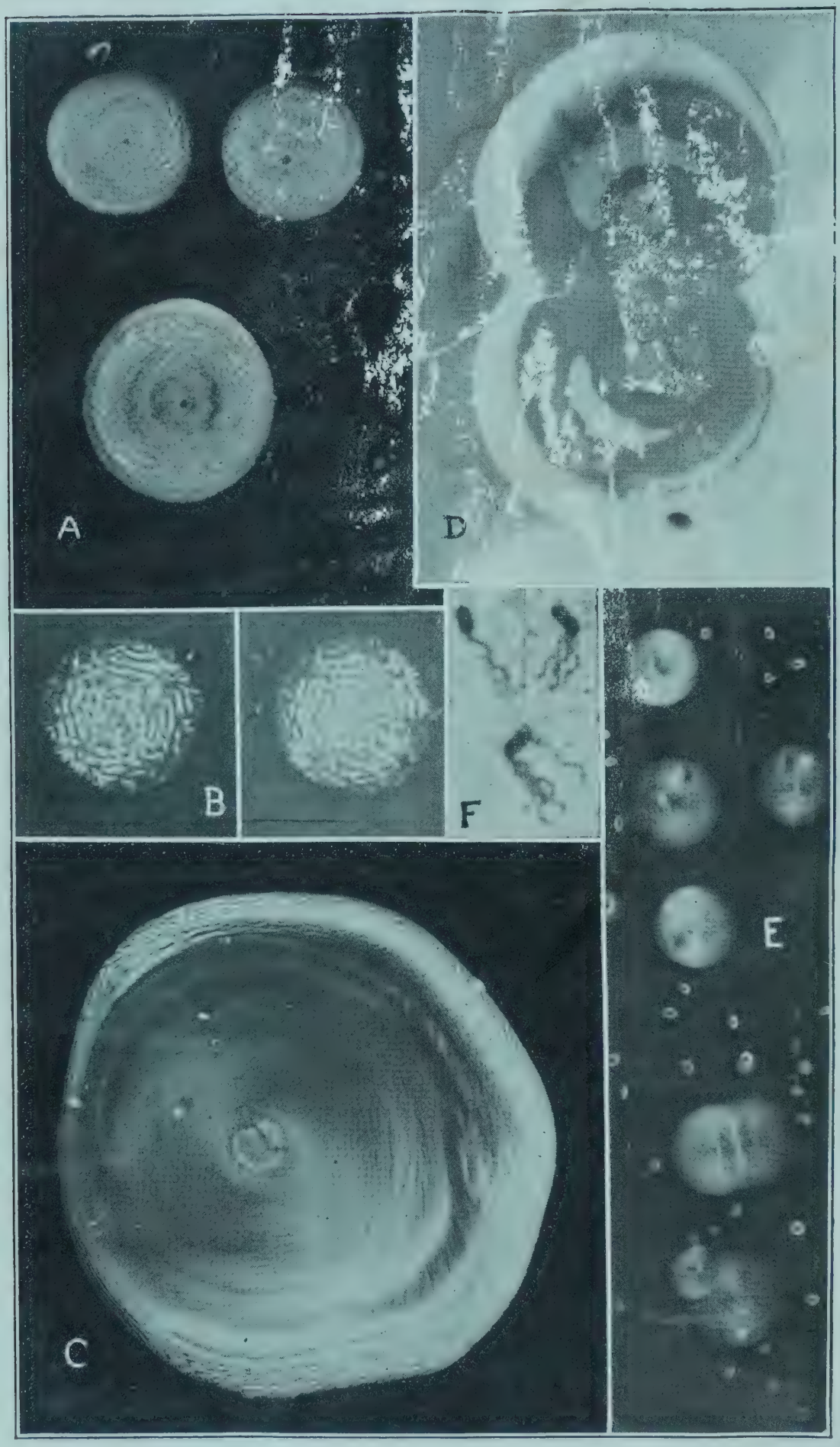

A
D
B
F
E
C

PLATE 63

Various types of colonies of *Bacterium atrofaciens:*

A.—Moderately crowded colonies on agar plate, 3 days old. Oblique light. ×10.

B.—Well-isolated colonies on agar plate, 2 days old. Oblique transmitted light. ×10.

C.—Single colony on agar plate, 20 days old. Oblique transmitted light. ×6.

D.—Well-isolated colonies on agar plate, 3 days old. Direct transmitted light. ×10.

E.—Crowded colonies on agar plate, 2 days old. Oblique transmitted light. ×10.

A and E represent the ordinary type of young colony. B and D are atypical.

F.—Flagella (Van Ermengem's stain). Stained by M. K. Bryan. Photographed by Erwin F. Smith. About ×2,000.

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EFFECT OF THE RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS

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INTRODUCTION

The importance of the relationships existing between light and plant growth and development has been so long recognized and these relationships have been of so much interest to investigators that a very extensive literature on the subject has been developed. For present purposes it will not be necessary to attempt even a brief review of this literature, and only some of the leading features bearing upon the particular problems in hand need to be touched upon. For more extended discussions of the work in this field the monographs of MacDougal (*18*)[2] and Wiesner (*26*) may be consulted. Three primary factors enter into the action of light upon plants—namely, (1) the intensity of the light, (2) the quality, that is, the wave length of the radiation, and (3) the duration of the exposure. Most phases of these three factors have been more or less extensively investigated. In the present investigation we are concerned chiefly with the general growth and development of plants and the reproductive processes as affected by the daily duration of the light exposure.

As regards intensity, it seems to be pretty well established that there is an optimum for growth in each species and that for many species this optimum is less than the intensity of the full sunlight on a clear day. Within limits, reduction in light intensity tends to lengthen the main

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[2] Reference is made by number (italic) to "Literature cited," p. 605-606.

axis and branches and to increase the superficial area of the foliage of many species. Also, the thickness of the leaf lamina may be reduced, and there may be marked departures from the normal in internal structure, the tendency being toward a less compact structure. So far as is known, no important general relationships between differences in light intensity and reproductive processes have been experimentally demonstrated.

The comparative effects produced by different regions of the spectrum, including the ultra-violet, have been extensively investigated but with more or less conflicting results. The most extensive investigations on the subject, perhaps, have been made by Flammarion (*8*). It was found that there is abnormal elongation of the principal axis in several species under the influence of the red rays, while growth is markedly reduced under the green and especially under the blue rays. In some plants, however, such as corn, peas, and beans, growth is greatest in white light. Some plants blossomed considerably earlier in red light than in white. White light produced the greatest weight of dry matter. Leaves of Coleus developed decided differences in color patterns under differently colored lights. In subsequent work Flammarion has extended his studies to a large number of species.

The duration of the daily exposure to light needs to be considered in three separate phases—(1) continuous illumination throughout the 24-hour period, (2) continuous darkness throughout, leading to the phenomena of etiolation, and (3) illumination for any fractional portion of the 24-hour day. Under natural conditions continuous sunlight throughout the 24-hour period occurs, of course, only in very high latitudes. Schübeler (*23*) observed the behavior of several species transported from lower latitudes and grown in northern Scandinavia under continuous sunlight lasting for a period of two months. In the species under observation the vegetative period was shortened and the seeds produced were larger than the normal. It is stated, also, that there was an increased formation of aromatic and flavoring constituents. Another method of securing continuous illumination consists in the use of artificial light for illumination or in the supplementing of normal daylight with artificial light, though, of course, the quality and the intensity from the two sources will not ordinarily be the same. Using electric light alone, of an intensity one-third that of sunlight, Bonnier (*6*) observed a marked increase in chlorophyll formation which extended inwardly to unusual depths. He found also incomplete differentiation of the tissues, recalling, in this respect, the effects of continued darkness. In some instances the color of blossoms was deepened.

Etiolation, resulting from exposure to continuous darkness, has been the subject of much study. In this connection special mention should be made of the work of MacDougal (*18*) covering a very large number of species. This author also presents a comprehensive survey of previous

work on the subject. In most instances stems, and frequently leaves, exhibited negative geotropism in the absence of light. In all species investigated etiolated tissues show a lesser degree of differentiation than the normal. In this connection MacDougal points out that the differences exhibited between etiolated specimens and normal plants demonstrate the fact that growth, or increase in size, and development, or differentiation, are distinct processes capable of separation. For present purposes perhaps his most important observation is that in no plant investigated had the stamens and pistils attained functional maturity.

The effects of differences in the length of the daylight period, the subject of the present study, have not been so extensively investigated as most other phases of light action. Obviously the problem may be approached in any one of four ways: by comparing the behavior of plants when propagated in different latitudes, by growing plants at different seasons of the year in the same latitude, by supplementing the daylight period with artificial light, and by preventing light from reaching the plant for a portion of the normal daylight period. In the records of attempts to grow various plants in different parts of the world there are undoubtedly a great deal of available data bearing on the present problem; but apparently no systematic effort has been made to utilize this material, the reason probably being that the importance of the relative length of day in affecting plant processes, and, in particular, reproduction, has not been appreciated. Bailey (*3*, *4*, *5*,) carried out an extensive series of tests in which daylight illumination was supplemented by the electric arc light applied for different portions of the night. The addition of the artificial light induced blossoming and seed formation in spinach. The additional light also favored the growth of lettuce. Rane (*20*), using the incandescent filament electric light, and Corbett (*7*), employing incandescent gas light, observed that certain flowering plants and some vegetables blossomed somewhat earlier when the normal daylight illumination was supplemented with artificial light. In most of these tests the artificial light was applied for the entire night, but apparently the results so far as concerns reproduction were essentially the same as when the plants were darkened for a portion of the night. Tournois (*24*, *25*) has reported the results of an interesting experiment with hemp (*Cannabis sativa* L.) and a species of hops (*Humulus japonicus* Sieb. and Zucc.) in which these plants were exposed to sunlight only from 8 a. m. to 2 p. m. daily. It had been shown by Girou de Buzareingues (*10*) as early as 1831 that when planted in the late winter or very early spring months the hemp plant first develops in the spring a number of abnormal sterile blossoms in the leaf axils and later produces normal flowers at the regular blossoming period. Following up this fact Tournois concludes from the above-mentioned experiment that the abnormal

blossoming period is induced by the short length of day prevailing in the early spring months.

In a few words, previous work on light action clearly indicates that permanent exclusion of light effectually prevents completion of the blossoming and seed-forming processes, while in certain cases lengthening the normal daily period of illumination by the use of artificial light or by propagation in far northern latitudes hastens the approach of the blossoming period, and, in the case of two species, shortening the daily exposure to light induces the formation of precocious blossoms. That the relative length of the day is really a dominating factor in plant reproduction processes, as is demonstrated in the present paper, seems not to have been suspected by previous workers in this field.

PRELIMINARY OBSERVATIONS

In 1906 there were observed in a strain of Maryland Narrowleaf tobacco (*Nicotiana tabacum*, L.), which is a very old variety, several plants which grew to an extraordinary height and produced an abnormally large number of leaves. As these plants showed no signs of blossoming with the advent of cold weather, some of them were transplanted from the field to the greenhouse and the stalks of others were cut off and the stumps replanted in the greenhouse. These roots soon developed new shoots which blossomed and produced seed, as did also the plants which had been transferred in their entirety. This very interesting giant tobacco, commonly known as Maryland Mammoth, which normally continues to grow till cold weather in the latitude of Washington, D. C., without blossoming, proved to be a very valuable new type for commercial purposes, but the above-mentioned procedure has been the only method by which seed could be obtained. The type bred true from the outset, and no matter how small the seed plant the progeny have always shown the giant type of growth when propagated under favorable summer conditions. It may be remarked at this point that inheritance of gigantism [1] in this tobacco has been studied by one of the present writers (*2*) and it has been shown that this character acts as a simple Mendelian recessive.

On one occasion it was observed that seedlings of the Mammoth transplanted to 8-inch pots in late winter blossomed in early spring after reaching a height of some 3 feet and developed an excellent crop of seed. From this it was at first concluded that growing the plant under conditions of partial starvation would induce blossoming, but this idea proved to be erroneous. Repeated attempts during the summer months to force blossoming by subjecting the plant to conditions which would permit only limited growth were futile. On the other hand, it was found that

[1] Throughout this paper the term gigantism is used to signify a tendency toward more or less indefinite vegetative activity manifested by plants under certain favorable environmental conditions. Though an inherited characteristic, it may come into expression only under definite conditions of environment; and the present investigation seems to make it clear that the length of the daily light exposure is the controlling factor.

seedlings grown in the greenhouse during the winter months invariably blossomed without regard to the size of the pot containing the seedling or the extent to which the plant was stunted by unfavorable nutrition conditions. The seedlings behaved, therefore, like the summer-grown giant plants which were transferred to the greenhouse late in the fall. Finally, it was observed that the shoots which were constantly developing from the transplanted roots of giant plants transferred to the greenhouse blossomed freely during the winter months, but as early spring advanced blossoming soon ceased and the younger shoots once more developed giant stalks. Obviously, then, the time of year in which the Mammoth tobacco develops determines whether the growth is of the giant character. During the summer months the plants may attain a height of 10 to 15 feet or more and produce many times the normal number of leaves without blossoming, while during the winter months blossoming invariably occurs before the plants attain a height of 5 feet. Naturally it became of interest from both a practical and a scientific standpoint to determine the factor of the environment responsible for the remarkable winter effect in forcing blossoming. It may be added just here that gigantism also has been observed in several distinct varieties of tobacco other than the Maryland—namely, in Sumatra, Cuban, and Connecticut Havana.

Again, in following out an investigation on the relation of the nutrition conditions to the quantity of oil formed in the seeds of such plants as cotton, peanuts, and soybeans, the present writers (*9*) had occasion to investigate the significance of the observation made by Mooers (*19*), that successive plantings of certain varieties of soybeans (*Soja max* (L.) Piper) made through the summer months, show a decided tendency to blossom at approximately the same date regardless of the date of planting. In other words, the later the planting the shorter is the period of growth up to the time of blossoming. In the course of the investigation on oil formation it became desirable to study the possible effects of temperature differences on the process. Since it is much simpler and cheaper to maintain temperature differences during the winter by the use of heat than during the summer by means of refrigeration, it was planned to make some tests with soybeans during the winter. It was soon found, however, that the plants began to develop blossoms before they had made anything like a normal growth, and the few blossoms produced were cleistogamous, so that it became necessary to abandon the plan of conducting the tests in question during the winter months. As is the case with the Mammoth tobacco, the time of year in which the plants are grown exerts a very profound influence on growth and reproduction in the soybean.

In seeking a solution of the problem as to why the behavior of these plants is radically different from the normal during the fall and winter months one naturally thinks of light and temperature as possible factors. It was observed, however, that both the Mammoth tobacco and the

soybeans still showed the abnormal behavior in the winter even when the temperature in the greenhouse was kept quite as high as prevails out of doors during the summer months. This observation seemed to dispose of temperature as a possible factor of importance in the "winter effect." It is clear that the quantity of solar radiation received by plants is less in winter than in summer, for both the number of hours of sunshine per day and the intensity of the light are reduced during the winter months. The quality of the light also is affected, since the angle of elevation of the sun's path during the winter is less than during the summer and the selective absorptive action of the atmosphere comes into play. It happened that in the investigation on oil formation in seeds a number of experiments had been made with soybeans to determine the effect of light intensity on this process and, incidentally, it was observed that in no case was the date of blossoming materially affected by the intensity of the light. It had been found, also, that partial shading was without decided effect on the blossoming of the Mammoth tobacco. In view of these experiences it hardly seemed likely that the other primary factor controlling the maximum amount of radiation received by the plant—namely, the length of the daily exposure—could be responsible for the effects in question. Nevertheless, the simple expedient of shortening artificially by a few hours the length of the daily exposure to the sun by use of a dark chamber was tried, and some very striking results were obtained, as detailed in the following paragraphs.

PLAN OF THE EXPERIMENTS

The first experiments with the use of the dark chamber were begun in July, 1918. A small, ventilated, dark chamber with a door which could be tightly closed was placed in the field. The soybeans used in the tests were grown in wooden boxes 10 inches wide, 10 inches deep, and 3 feet long. These containers have been extensively used in growing soybeans and other small plants under controlled conditions, and it has been found that normal plants are easily obtained in this way. The dark chamber and the type of box used for growing soybeans and similar plants are shown in Plate 64, A. Larger plants like tobacco have been grown in large galvanized iron buckets or, in some cases, in ordinary flower pots. When the test plants have attained the desired stage of development the procedure has been to place them in the dark chamber at the selected hour in the afternoon each day. The plants were left in the dark chamber till the hour decided upon in the following morning, when they were again placed in the sunlight. This procedure was followed each day till the test was completed. Appropriate control plants were left in the open throughout the test in each case. By this method the number of hours of exposure to sunlight during the 24-hour period could be reduced as far as desired.

In the preliminary tests of 1918 no special means were provided for moving the boxes and pots containing the plants in and out of the dark chamber. In the spring of the present year a much larger dark house was constructed, and suitable facilities were installed for easily moving the test plants in or out of the house as often as desired. The dark house consisted of a rectangular frame structure 30 feet by 18 feet and 6 feet in height to the eaves and 9 feet to the ridgepole. All crevices by which light could enter were covered, tight-fitting doors were provided, and the interior was painted black. Means were provided at the bottom and top of the house for free circulation of air without the admission of light. A series of four steel tracks, each entering through a separate door, was provided; and on these tracks were mounted a number of trucks carrying the test plants in their containers. This equipment proved very satisfactory. A general view of the dark house, the trucks, and the test plants is shown in Plate 64, B.

It has been rather generally assumed that the pronounced changes in plant activities which come on with the approach of fall are due in some way to the lower mean daily temperatures or the wider daily range in temperature caused by cool nights. It seemed desirable, therefore, to compare the temperatures inside and outside the dark house, and for this purpose thermographs were installed. It was found that there were only slight differences in temperature. The temperature inside the dark house tended to run 2° or 3° F. higher than the temperature outside, particularly at night. Hence, any responses on the part of the plants resembling those appearing in the fall of the year could not be attributed to lower temperatures. To guard further against possible temperature effects, as soon as the above-mentioned temperature difference was discovered all doors of the dark house were opened as darkness came on each day.

In the various tests the length of the exposure to light was varied from a minimum of 5 hours per day to a maximum of 12 hours, 7 hours and 12 hours being the exposures chiefly used. For the shortest exposure the plants were placed in the dark house at 3 o'clock p. m. and returned to the light at 10 a. m.; for the 7-hour exposure the plants were darkened at 4 p. m. and returned to the light at 9 a. m.; and for the 12-hour exposure they were in the dark house from 6 p. m. till 6 a. m. A further modification in exposure consisted in placing the plants in the dark house at 10 a. m. and returning them to the light at 2 p. m. In most instances the daily treatment began with the germination of the seed or in the earlier stages of growth and continued until maturity, but in some cases the plants were permanently restored to the open as soon as blossoming occurred, and in other cases the artificial shortening of the day was not begun until after blossoming had occurred. To facilitate discussion it will be convenient to use the expressions "long day" as meaning exposure to light for more than 12 hours and "short day" as referring to an exposure of 12 hours or less. The term "length of day"

as used in this paper refers to the duration of the illumination period for each 24-hour interval.

As a part of the present investigation a series of plantings of soybeans was made in the field at intervals of approximately three days throughout the season, in order that the effects produced by different dates of planting might be compared with those produced by artificially shortening the length of the daily exposure to light.

BEHAVIOR OF THE PLANTS TESTED

The initiai experiment was made in the summer of 1918, and in this instance a box containing the Peking variety of soybeans in blossom and three pots containing Mammoth tobacco plants which had been growing for several weeks were first placed in the dark chamber at 4 p. m. on July 10 and removed therefrom at 9 a. m. the following morning. This treatment was continued each day till the seeds of the beans and tobacco were mature. All subsequent experiments were made during the year 1919. Details of the tests for both years follow.

SOYBEANS (SOJA MAX (L.) PIPER)

(a) MANDARIN [1] (F. S. P. I. No. 36,653), early maturing:

(1) Exposed to light from 10 a. m. to 3 p. m. Planted May 8, up May 17, and placed in dark house May 20. First blossoms appeared June 12 on test plants and June 15 on controls. Average height of test plants 6 to 7 inches and that of controls 18 to 20 inches. After blossoming, the growth and development of the seed pods was much more rapid in the test plants than in the controls.

(2) Exposed to light from 9 a. m. to 4 p. m. Planted May 8, up May 17, and placed in dark house May 20. First blossoms appeared June 10 on test plants and June 15 on controls. Average height of test plants 9 to 10 inches and that of controls 19 to 20 inches.

(3) Exposed to light from 6 a. m. to 6 p. m. Planted and placed in dark house June 11, up June 16. First blossoms appeared July 7 on test plants and July 14 on controls. Average height of test plants 14 to 15 inches and that of controls 32 to 33 inches. Six weeks after blossoming the seed pods and foliage were still green and the plants stocky, whereas, under the same conditions, the Peking variety, listed below, showed many brown, mature pods, foliage yellowing, and the plants slender.

(b) PEKING [1] (F. S. P. I. No. 32,907), medium maturing:

(1) Exposed to light from 10 a. m. to 3 p. m. Planted May 8, up May 17, and placed in dark house May 20. First blossoms appeared June 12 on test plants and July 21 on controls. Seed pods on test plants

[1] Horticultural variety.

were turning brown by July 18, and all were mature before August 10. Average height of test plants 5 to 6 inches and that of controls 42 to 43 inches. Test plants were restored to normal light exposure June 20.

(2) Exposed to light from 9 a. m. to 4 p. m. Planted May 8, up May 17, and placed in dark house May 20. First blossoms appeared June 10 on test plants and July 21 on controls. Average height of test plants 8 inches and that of controls 45 to 48 inches. See Plate 65.

(2a) Exposed to light from 9 a. m. to 4 p. m. Planted May 8, up May 17, placed in dark house June 7. First blossoms June 29. Average height of plants 16 to 17 inches.

(3) Exposed to light from 9 a. m. to 4 p. m. after blossoming. Planted May 7, blossomed July 9, and first placed in dark house July 10. By July 26 there were many full-grown pods on test plants while there were none on controls more than half-grown. By August 29 the leaves had yellowed and were falling, and some pods were fully ripe on test plants while control plants were still green. By September 7 all seeds were fully ripe on test plants, but those on controls did not fully mature till about October 1. See Plate 66.

(4) Exposed to light from 6 a. m. to 6 p. m. Planted and placed in dark house June 11, up June 16. First blossoms July 7 on test plants and August 6 on controls. Average height of test plants 14 to 15 inches and that of controls 39 to 40 inches.

(5) Exposed to light from daylight to 10 a. m. and from 2 p. m. till dark. Planted June 14, up June 19, and placed in dark house June 19. First blossoms July 29 on test plants and August 11 on controls. Average height of test plants 25 to 26 inches and that of controls 41 to 42 inches.

(c) Tokyo,[1] late maturing:

(1) Exposed to light from 10 a. m. to 3 p. m. Planted May 8, up May 17, and placed in dark house May 20. First blossoms appeared June 13 on test plants and July 29 on controls. Average height of test plants 7 to 8 inches and that of controls 49 to 50 inches. Test plants were restored to normal light exposure June 20.

(2) Exposed to light from 9 a. m. to 4 p. m. Planted May 8, up May 17, and placed in dark house May 20. First blossoms appeared June 13 on test plants and July 29 on controls. Average height of test plants 7 to 8 inches and that of controls 49 to 50 inches.

(2a) Exposed to light from 9 a. m. to 4 p. m. Planted May 8, up May 17, and placed in dark house June 7. First blossoms appeared July 4. Average height of plants 23 to 24 inches.

(3) Exposed to light from 6 a. m. to 6 p. m. Planted and placed in dark house June 11, up June 16. First blossoms appeared July 14 on test test plants and August 21 on controls. Average height of test plants 17 to 18 inches and that of controls 42 to 43 inches.

[1] Horticultural variety.

(4) Exposed to light from daylight till 10 a. m. and from 2 p. m. to darkness. Planted June 14, placed in dark house June 16, up June 19. First blossoms appeared August 20 on test plants and August 23 on controls. Average height of test plants 24 to 25 inches and that of controls 42 to 43 inches.

(d) BILOXI,[1] very late maturing:

(1) Exposed to light from 10 a. m. to 3 p. m. Planted May 8, up May 17, and placed in dark house May 20. First blossoms appeared June 16 on test plants and September 4 on controls. Average height of test plants 6 to 7 inches and that of controls 57 to 58 inches. See Plate 68, A. Test plants were restored to normal light exposure June 20.

(2) Exposed to light from 9 a. m. to 4 p. m. Planted May 8, up May 17, and placed in dark house May 20. First blossoms appeared June 15 on test plants and September 4 on controls. Average height of test plants 11 inches and that of controls 57 to 58 inches. See Plate 67.

(2a) Exposed to light from 9 a. m. to 4 p. m. Planted June 10, up June 15, and placed in dark house June 24. First blossoms July 22 on test plants and September 15 on controls. Average height of test plants 15 to 16 inches and that of controls 56 to 58 inches.

(3) Exposed to light from 6 a. m. to 6 p. m. Planted and placed in dark house June 11, up June 16. First blossoms appeared July 14 on test plants and September 8 on controls. Average height of test plants 23 to 24 inches and that of controls 54 to 55 inches. See Plate 68, B.

(4) Exposed to light from daylight to 10 a. m. and from 2 p. m. to darkness. Planted June 14, placed in dark house June 16, up June 19. First blossoms appeared September 6 on test plants and September 15 on controls. See Plate 69, A. Average height of test plants 39 to 40 inches and that of controls 47 to 48 inches. In all of the above-described tests with soybeans observations were made on from 20 to 25 individuals.

TOBACCO (NICOTIANA TABACUM AND N. RUSTICA L.)

(a) NICOTIANA TABACUM;[1] MARYLAND MAMMOTH, giant type:

(1) Exposed to light from 10 a. m. to 3 p. m. Observations on 14 test plants and 10 controls. Planted March 6, transplanted to 6-inch pots May 10, and placed in dark house May 14. First blossoms appeared July 8 to August 14 on test plants and in last week of October on controls. Average height of test plants 14 to 16 inches and that of controls 3 to 5 inches.

(2) Exposed to light from 9 a. m. to 4 p. m. Observations on 7 test plants and 10 controls. Planted March 6, transplanted to 6-inch pots May 10, and placed in dark house May 14. First blossoms appeared July 18 to August 1 on test plants and in last week of October on controls.

[1] Horticultural variety.

Average height of test plants 12 to 14 inches and that of controls 5 to 6 inches.

(2a) Exposed to light from 9 a. m. to 4 p. m. Observations on 8 test plants and 8 controls. Planted January 8, transplanted to 8-inch pots May 3, and placed in dark house May 14. First blossoms appeared July 5 to 25 on test plants and October 1 to 25 on controls. See Plate 70.

(2b) Exposed to light from 9 a. m. to 4 p. m. Obseravtions on three test plants and four controls. Planted April 14, transplanted in steam-sterilized soil in 12-quart iron pails and placed in dark house June 10. First blossoms appeared August 1 to 7 on test plants and August 30 to September 8 on controls. Average height of test plants 37 inches and that of controls 39 inches.

(3) Exposed to light from 6 a. m. to 6 p. m. Observations on 6 test plants and 3 controls. Planted April 14 and transplanted to 12-quart iron pails containing steam-sterilized soil and placed in dark house June 11. First blossoms appeared August 26 to September 4 on test plants and September 3 to 20 on controls. Average height of test plants 48 inches and that of controls 49 inches. See Plates 71 and 72, A.

(b) N. TABACUM; STEWART 70-LEAF CUBAN,[1] giant type:

(1) Exposed to light from 9 a. m. to 4 p. m. Observations on 6 test plants and 5 controls. Planted April 14 and transplanted in steam-sterilized soil in 12-quart iron pails and placed in dark house June 10. First blossoms appeared August 16 to September 2 on test plants and September 24 to October 10 on controls. Average height of test plants 53 to 69 inches and that of controls 73 to 84 inches.

(c) N. TABACUM; CONNECTICUT BROADLEAF:[1]

(1) Exposed to light from 9 a. m. to 4 p. m. Observations on 11 test plants and 10 controls. Planted April 14 and transplanted to 14-quart iron pails and placed in dark house June 5. First blossoms appeared July 18 to 24 on test plants and July 17 to 22 on controls. Average height of test plants 38 inches and that of controls 34 inches. Average number of nodes on test plants 36 and same number on controls.

(1a) Exposed to light from 9 a. m. to 4 p. m. Observations on 8 test plants and 6 controls. Planted April 5 and transplanted to 14-quart iron pails and placed in dark house May 28. First blossoms appeared July 13 to 20 on test plants and July 7 to 15 on controls. Average height of test plants 37 inches and that of controls 40 inches.

(d) N. RUSTICA:

(1) Exposed to light from 9 a. m. to 4 p. m. Observations on 5 test plants and 3 controls. Planted April 14, transplanted to 14-quart iron pails, and 5 plants placed in dark house on June 2. Test plants blossomed July 5 to 28 and controls July 1 to 12.

[1] Horticultural variety.

ASTER LINARIIFOLIUS L.

A common wild aster found in dry, open situations from Maine to Wisconsin and southward. The normal blossoming period begins about September 1 and extends over a period of two or three months.

(1) Exposed to light from 9 a. m. to 4 p. m. Six individuals taken from the field May 13 and transplanted to boxes of the type used for soybeans, three plants to the box. One box of the plants placed at once in the dark house. The control plants soon resumed vegetative development, throwing out numerous axillary branches on the upper portion of the stems as the normal limit in height was approached, thus following the regular course of development in the field. The test plants, on the other hand, made little additional growth and by June 1 were showing tiny flower heads. First blossoms appeared June 18 on test plants and September 12 on controls. Average height of test plants on June 24, 8 to 10 inches and that of controls 14 to 15 inches. Test plants were permanently returned to normal light on June 20. See Plate 72, B.

(2) Exposed to light from 6 a. m. to 6 p. m. Three individuals transplanted from field to each of two 8-gallon iron cans June 10 and those in one can placed in dark house June 12. Tiny flower heads were showing on the test plants by July 2. First blossoms appeared July 19 on test plants and September 20 on controls. Average height of test plants 8 to 9 inches and that of controls 14 to 15 inches.

(3) Exposed to light from daylight to 10 a. m. and from 2 p. m. to darkness. Three individuals transplanted from the field to each of two 8-gallon iron cans on June 14 and those in one can placed in dark house June 16. Flower heads were showing on both test plants and controls by August 20. First blossoms appeared September 16 on test plants and September 18 on controls. Average height of test plants 11 to 12 inches and that of controls 14 to 15 inches.

CLIMBING HEMPWEED (MIKANIA SCANDENS, L.)

A climbing composite, ranging from southern Maine to Florida and westward to Ontario, Mississippi, and Texas. The normal blooming period extends from late July to the latter part of September. The aerial summer growth perishes in the fall, and the plants are carried over the winter period by perennial underground shoots.

(1) Exposed to light from 9 a. m. to 4 p. m. A number of roots were transplanted from the field to 6-inch pots and placed in the greenhouse in November, 1918. These roots threw up shoots which made considerable growth during the winter months but did not blossom. On June 3 one plant was transferred to each of six 12-quart iron pails, three of which were placed in the dark house at once. The controls began blossoming in late July and continued to blossom profusely till the latter part of September. Some of the plants which had been left in the green-

house, where the temperature was much higher than out-of-doors, blossomed at the same time. The test plants behaved quite differently, for blossoming was completely inhibited throughout the summer. Moreover, the growth of the controls has been considerably greater than that of the test plants. See Plate 74.

BEANS (PHASEOLUS VULGARIS L.)

Three lots of seed of a tropical bean—two of which came from Arequipa, Peru, and one from Oruro, Bolivia—were planted together in two boxes measuring 3 feet by 10 inches by 10 inches on June 16, and one box was placed in the dark house June 24. Exposed to light from 9 a. m. to 4 p. m. According to Dr. D. N. Shoemaker this bean when planted in the field at Washington has been found to make a very large growth without blossoming till late in the fall, but when propagated in the greenhouse in the winter months the plant promptly blossoms and sets seed. The test plants blossomed July 21 to 23, and some of the seed pods were mature by August 22, whereas the controls did not blossom till October 11. The average height of the test plants was 4½ to 5 feet and that of the controls 7 to 8 feet. See Plate 73.

RAGWEED (AMBROSIA ARTEMISIIFOLIA L.)

Exposed to light from 9 a. m. to 4 p. m. Observations based on 6 test plants and 6 controls. Small plants taken from the roadside were transplanted to 6-inch pots on June 3, and a portion of these were immediately placed in the dark house. Staminate heads were showing on the test plants by June 17, and the anthers were shedding pollen freely by July 1. The controls did not begin blossoming till the last week in August, which is the normal period for the appearance of first blossoms on the plant. The average height of the test plants at the time of blossoming was 8 to 9 inches, while that of the controls on the same date was 11 inches and their final height 29 inches. The test plants were returned permanently to normal light exposure on July 1. See Plate 75, A.

RADISH (RAPHANUS SATIVUS L.)

SCARLET GLOBE:[1]

Exposed to light from 9 a. m. to 4 p. m. Planted May 15, up May 19, and placed in dark house on day of planting. The test plants grew more slowly than the controls for a time and then appeared to grow no further. All but two of the test plants, of which there were a large number, became diseased and finally died without forming seed stalks. The two survivors developed a crown of large leaves, and the roots also reached much larger proportions than those of the controls. Apparently enlargement of the roots had not ceased as late as October 15, when one

[1] Horticultural variety.

of them measured nearly 4 inches in diameter while its rosette of leaves measured 30 inches from tip to tip. Flower stems did not develop. The controls grew more rapidly from the outset, and all except three or four to be considered later formed flower stems in June, the first blossom appearing June 21. See Plate 75, B.

CARROT (DAUCUS CAROTA L.)

OXHEART:[1]

Exposed to light from 9 a. m. to 4 p. m. Planted June 4 and at once placed in dark house. The test plants made a uniform but slow growth, and the roots, which were very small, appeared to be devoid of the yellow pigment, carotin, since they were almost snow-white in color. The controls grew and developed normally, the roots showing the normal yellow color. On August 19 the average height of the test plants was 8 to 9 inches and that of the controls 18 to 20 inches. See Plate 79, B.

LETTUCE (LACTUCA SATIVA L.)

BLACK SEEDED SUMMER:[1]

Exposed to light from 9 a. m. to 4 p. m. Planted in dark house June 4. Germination was satisfactory, but the seedlings made very little growth, and after a time all died. The controls grew vigorously but under the stimulus of the long day the plants soon sent up flowering shoots and blossomed.

HIBISCUS MOSCHEUTOS L.

A wild perennial in marshes, ranging from Ontario to Florida and Texas. Normal blooming period July to September. Exposed to light from 9 a. m. to 4 p. m. Planted in November in greenhouse. Seed did not germinate till the following March. Seven plants transferred to 12-quart iron pails on June 6, three of which were placed in dark house June 7. The test plants did not blossom nor did they make any growth during the summer. The controls grew vigorously, and the first blossoms appeared August 22 to September 10. The average height of the test plants was 12 inches and that of the controls 29 inches.

CABBAGE (BRASSICA OLERACEA CAPITATA L.)

EARLY JERSEY WAKEFIELD:[1]

Exposed to light from 9 a. m. to 4 p. m. Observations based on four test plants and four controls. Transplanted and placed in dark house on June 7. The test plants grew slowly but uninterruptedly throughout the season, although they showed little tendency to form heads. The control plants grew normally and formed large heads which eventually burst open, followed by the formation of new heads of small size.

[1] Horticultural variety.

VIOLETS (VIOLA FIMBRIATULA SM.)

A common wild species ranging from Nova Scotia to Wisconsin and southward and growing in sandy fields and on dry hillsides. The normal blooming period comes in April. Exposed to light from 9 a. m. to 4 p. m. Two lots of six plants were transferred from the field to two boxes measuring 3 feet by 10 inches by 10 inches on June 9, and one of the boxes was placed at once in the dark house. The test plants showed flower buds as early as June 21 and were in blossom early in July, producing purple, petaliferous flowers and also cleistogamous flowers. The control plants produced numerous cleistogamous flowers but none of the purple, petaliferous type.

EARLY GOLDENROD (SOLIDAGO JUNCEA AIT.)

The earliest species of goldenrod, ranging from New Brunswick to Saskatchewan and south to North Carolina and Missouri. Blossoming normally extends from late June to September. Exposed to light from 9 a. m. to 4 p. m. Two lots of six plants were transplanted to two boxes measuring 3 feet by 10 inches by 10 inches on June 6, and one of the boxes was at once placed in the dark house. The test plants and the controls blossomed at the same time, late in August. The test plants however, were shorter and more compact than the controls. The heights of the test plants averaged 24 inches and those of the controls 38 inches. The test plants advanced toward maturation more rapidly than the controls after the flowering stage had been reached.

EFFECT OF RESTORING THE TEST PLANTS TO NORMAL LIGHT EXPOSURE AFTER BLOSSOMING HAD OCCURRED

In the experiments with soybeans, aster, and ragweed described above it has been made clear that after blossoming has occurred the effect of shortening the daily exposure to sunlight is to hasten greatly the ripening of the seed. In certain instances, however, as has been recorded under the several experiments, the test plants were restored to the normal light exposure as soon as blossoming had occurred.

Under these conditions seed pods of the soybeans ripened rapidly, the leaves turned yellow, and for a time it appeared that the plants would die as is normal for the soybean. Eventually, however, new branches developed under the influence of the long summer days. The renewed growth was especially well-developedin the Biloxi variety, and the final result was that these plants, still bearing the first crop of ripened seed pods, blossomed for the second time September 4 to 8. This date of blossoming, moreover, is also that for the first blossoming of the control plants which had been planted on the same date as the test plants and had been exposed to the normal daylight period throughout their development.

Like the soybeans, the asters after a time responded to the long-day influence; and by July 20 the plants, though bearing ripened seed, were

developing new axillary branches. The new growth finally developed flower heads; and thus the plants blossomed for the second time during the first half of September, which is the time of blossoming of the original

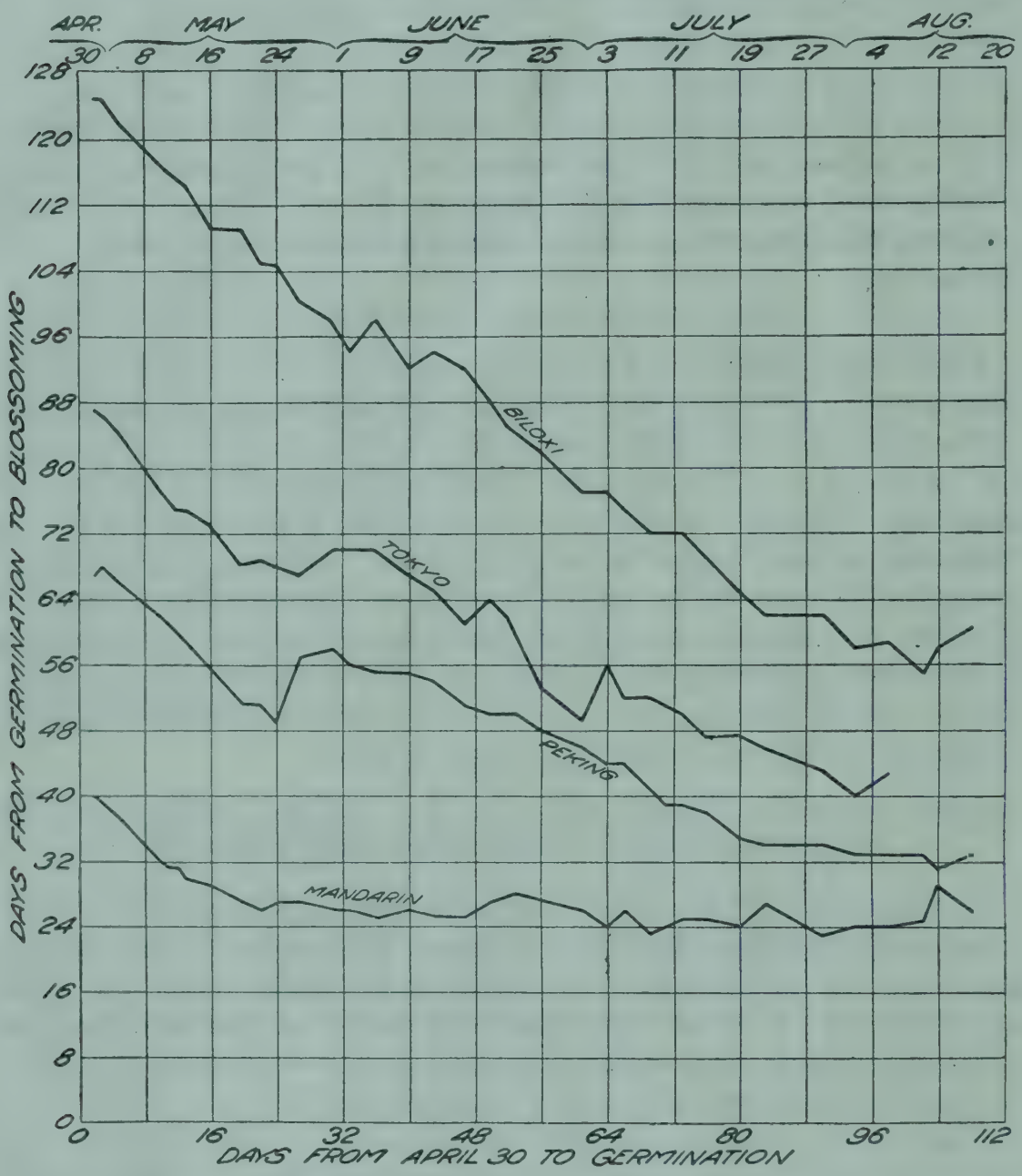
APR.
MAY
JUNE
JULY
AUG.
30 8 16 24 1 9 17 25 3 11 19 27 4 12 20
128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0
DAYS FROM GERMINATION TO BLOSSOMING
BILOXI
TOKYO
PEKING
MANDARIN
0 16 32 48 64 80 96 112
DAYS FROM APRIL 30 TO GERMINATION

FIG. 1.—Graph showing the shortening of the vegetative period preceding flowering in soybeans which results from progressively later planting during the growing season.

controls exposed to the normal daylight period throughout their development.

The ragweed, likewise, resumed vegetative development after a time, and, in fact, under the influence of the full length of the daylight period the new growth exceeded in size that of the original plants. The plants blossomed the second time during the last week in August, which is also the time of blossoming of the original controls and of ragweed growing in the field. It may be noted, however, that while the original growth produced staminate spikes as well as pistillate flowers in the usual man-

ner, the second growth produced pistillate flowers almost exclusively and the leaves were mostly atypical.

RELATION OF DATE OF PLANTING TO DATE OF BLOSSOMING IN SOYBEANS

Through the spring and summer of 1919 a series of plantings of soybeans which included the four varieties used in the tests described above were made in the field at regular intervals of three days as nearly as conditions would permit. All plantings of each variety consisted of rows 10 feet in length. The date recorded as that when first blossoms appeared is in each case that when the majority of the individuals in the planting first showed one or two open blossoms. In most instances the

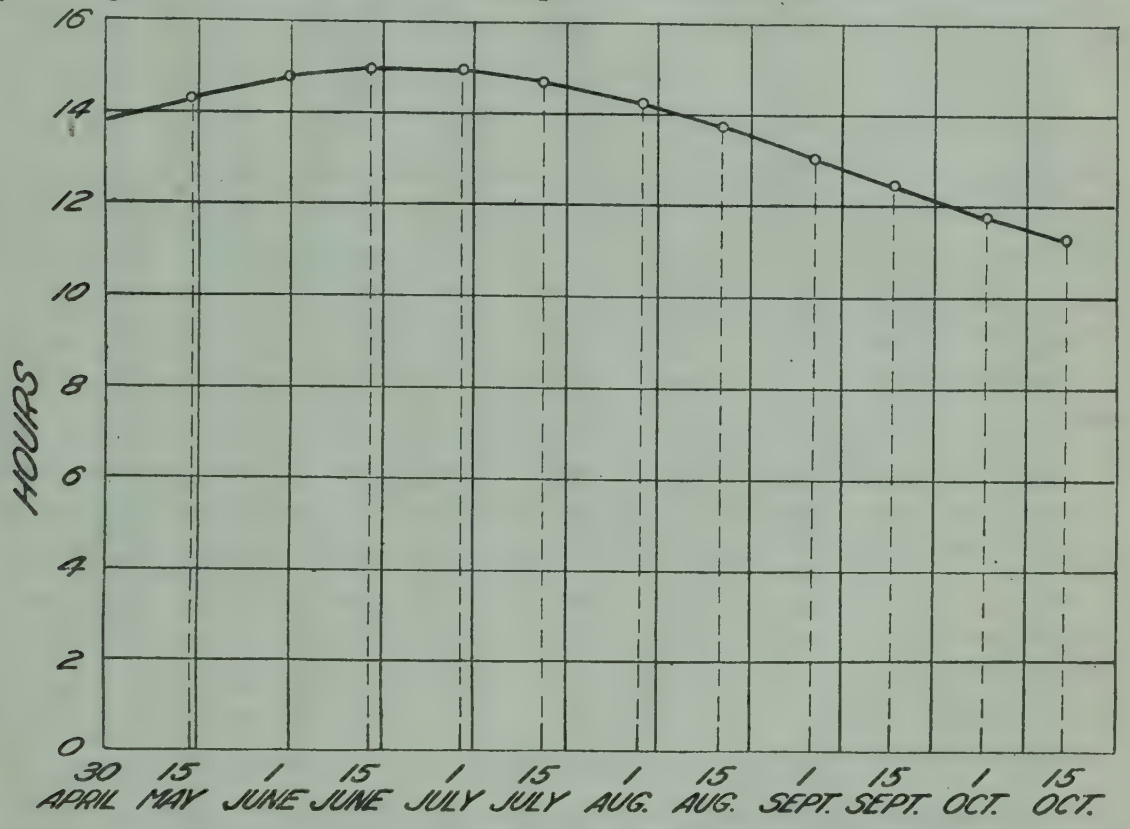

FIG. 2.—Graph showing changes in length of day during the growing season in the latitude of Washington, D. C. Ordinates indicate 2-hour intervals of the day and abscissae indicate 16-day periods of the growing season.

greater number of the individuals in a planting showed their first open blossoms on practically the same date. The dates of planting, germination, and appearance of first blossoms, together with the number of days from germination till blossoming are shown in Table I.

The effect of the date of planting on the length of the period from germination to the blossoming stage for each variety is more easily seen in the curves of figure 1, in the construction of which the number of days from April 30 to dates of germination are used as ordinates and the number of days included in the periods of growth prior to blossoming are used as abscissae. The relative length of the day—that is, the time between sunrise and sunset, expressed in 2-hour periods—also is shown for the same period in figure 2. The relative heights of the plants in the consecutive plantings of the Biloxi variety are shown graphically in figure 3.

TABLE I.—*Effect of date of planting on date of blossoming of soybeans grown in field at Arlington, Va., 1919*

Date of planting.	Date of appearance above ground.	Mandarin.		Peking.		Tokyo.		Biloxi.	
		Date of first blossoms.	Time from germination to blossoming.	Date of first blossoms.	Time from germination to blossoming.	Date of first blossoms.	Time from germination to blossoming.	Date of first blossoms.	Time from germination to blossoming.
			Days.		*Days.*		*Days.*		*Days.*
Apr. 9..	May 2	June 11	40	July 8	67	July 28	87	Sept. 4	125
14..	3	11	39	10	68	28	86	5	125
18..	5	11	37	10	66	28	84	4	122
22..	10	11	32	11	62	26	77	4	117
26..	11	11	31	11	61	26	76	4	116
30..	12	12	31	11	60	26	75	4	115
May 3..	13	12	30	11	59	27	75	4	114
6..	16	14	29	11	56	28	73	2	109
9..	20	16	27	11	51	27	68	6	109
13..	22	17	26	12	51	30	69	4	105
16..	24	20	27	12	49	31	68	6	105
20..	27	23	27	23	57	Aug. 2	67	4	100
24..	31	26	26	28	58	9	70	6	98
27..	June 2	28	26	28	56	11	70	4	94
31..	5	30	25	30	55	14	70	11	98
June 4..	9	July 5	26	Aug. 3	55	15	67	11	92
7..	12	7	25	5	54	16	65	10	94
11..	16	11	25	6	51	16	61	11	92
14..	19	16	27	8	50	22	64	15	88
17..	22	20	28	11	50	23	62	15	85
20..	25	22	27	12	48	17	53	15	82
23..	30	26	26	15	46	18	49	15	77
26..	July 3	27	24	16	44	26	56	18	77
30..	5	31	26	18	44	26	52	18	75
July 3..	8	31	23	18	41	29	52	18	72
7..	12	Aug. 6	25	20	39	31	50	22	72
10..	15	9	25	22	38	31	47	22	69
14..	19	12	24	23	35	Sept. 4	47	20	63
17..	22	18	27	25	34	6	46	22	62
25..	29	21	23	Sept. 6	39	10	43	29	62
29..	Aug. 2	26	24	6	35	11	40	29	58
Aug. 2..	6	30	24	8	33	.....	.....	Oct. 4	59
5..	10	Sept. 4	25	11	33	.....	.....	4	55
8..	12	10	29	12	31	.....	.....	9	58
11..	16	11	26	18	33	.....	.....	16	61
14..	17	.....	.....	19	33	.....	.....	.....	.....
20..	25	.....	.....	26	31	.....	.....	.....	.....

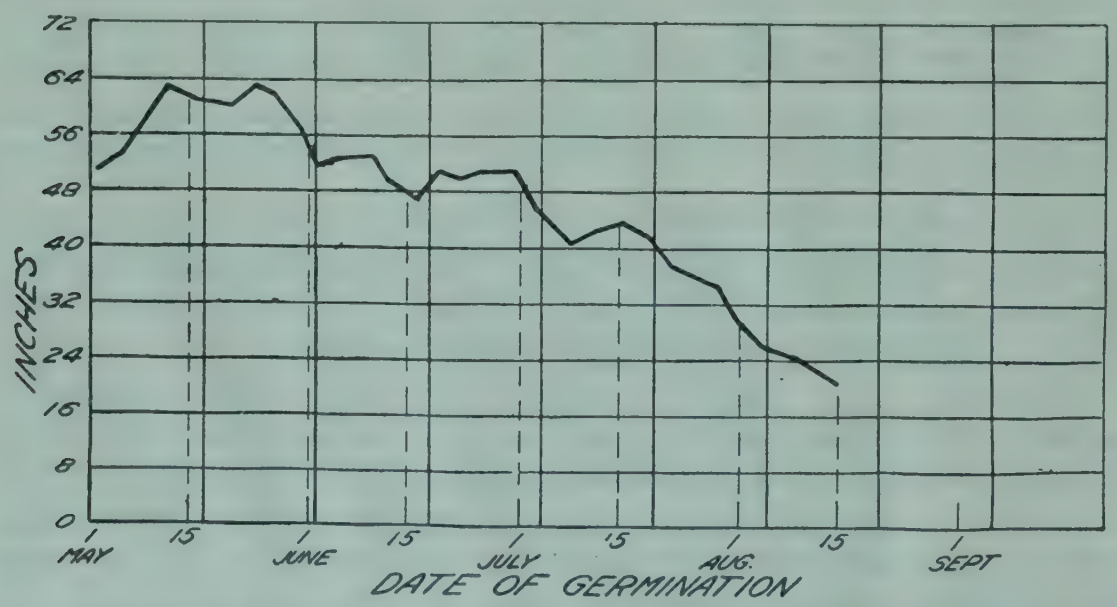

FIG. 3.—Graph showing the progressive decrease in height attained by Biloxi soybeans as the date of planting is delayed beyond late spring

DISCUSSION OF RESULTS

The results of the experiments which have been described show clearly that both the rate and extent of the growth attained by the plants under study and the time required for reaching and completing the flowering and fruiting stages are profoundly affected by the length of the daily exposure to sunlight. The behavior of some of the plants under the different exposures would seem to indicate that the action on the vegetative phase of development is more or less independent of that on reproduction, but only tentative conclusions can be drawn on these points at the present time. The effects of the different light exposures on these two phases of plant development can best be discussed separately.

LENGTH OF DAILY LIGHT EXPOSURE IN RELATION TO VEGETATIVE DEVELOPMENT

Under the conditions of the tests it was not possible to secure quantitative data on the various details of vegetative growth and development, but measurements of height and the photographic records will clearly indicate some of the differences resulting from the various light exposures. In general, the extent of growth was proportional to the length of the daily exposure to light; and this held true when the plants received two daily exposures to light, with an intervening period of darkening, as well as when there was only a single daily exposure to the light. Under the shorter exposures the plants were shorter and less stocky, and there were some indications of etiolation or chlorosis. Histological examination of the test plants was not undertaken, but in most species no very striking differences in gross anatomy resulted from the different exposures. Broadly speaking, the extent rather than the character of growth and vegetative development was chiefly affected. Table II is intended to bring out the relationship between size of plant and length of the exposure to the light for soybeans and the aster. This relationship is strikingly brought out for the Biloxi soybean in figure 3, which shows the decreasing heights of progressively later plantings. How length of exposure affects the Mandarin is shown in the foreground of Plate 78, B.

TABLE II.—*Effect of length of daily exposure to light on the height of soybeans and aster*

Length of daily exposure.	Average heights of plants.				
	Soybeans.				Aster.
	Mandarin.	Peking.	Tokyo.	Biloxi.	
	Inches.	*Inches.*	*Inches.*	*Inches.*	*Inches.*
10 a. m. to 3 p. m., 5 hours....	6 to 7	5 to 6	7 to 8	6 to 7	.....
9 a. m. to 4 p. m., 7 hours.....	9 to 10	8	7 to 8	11	8 to 10
Daylight to 10 a. m. and 2 p. m. till dark, 8½ to 11 hours[a]..	.....	25 to 26	24 to 25	38 to 40	11 to 12
6 a. m. to 6 p. m., 12 hours....	14 to 15	14 to 15	17 to 18	23 to 24	8 to 10
Full daylight, 12½ to 15 hours.	18 to 20	40 to 44	42 to 44	54 to 58	14 to 15

[a] The relatively greater heights in proportion to the number of hours in the total daily exposures under this treatment are due to the fact that in this case the length of the growing period wa not materially shortened by forced earliness in blossoming; they are not to be ascribed to an increased rate of growth.

It may be worthy of note that in the tests under controlled conditions the height of the Biloxi plants under a 12-hour light exposure was practically the same as that of the latest field plantings shown in figure 3, while that of the controls was about the same as that of the early field plantings.

Since in many cases the length of the growing period was greatly curtailed by the forcing action of reduced light exposure on reproduction, the amount of growth was necessarily limited thereby in those plants having a determinate type of inflorescence; but, in addition, measurements made when the blossoming stage of the forced plants had been reached show that the rate of growth was greater as the length of the exposure to light increased. The measurements of height recorded under the several tests relate to the final heights attained by the plants. In the species tested no exceptions to the foregoing principle were encountered; but it is possible, of course, that other species will be found to act differently. It has been demonstrated by a number of investigators that when many green plants are transferred from light to darkness the immediate effect is an acceleration in the rate of growth; and, conversely, the first effect of exposure to light is a retarding of growth. These facts, however, bear on necessary relation to the total effect on rate of growth over a considerable period of time produced by differences in the relative length of night and day.

It remains to be pointed out that striking differences in sensitiveness to decreased length of the daily exposure to light were observed in the different species under investigation. Aside from considerable reductions in the rate of growth and slight chlorosis, soybeans, tobacco, aster, and some others showed no ill effects from the reduced length of illumination, while Hibiscus was not able to make any appreciable growth with the illumination period reduced to nine hours, and lettuce was much more seriously affected, all individuals having perished without making any material growth.

LENGTH OF DAILY LIGHT EXPOSURE IN RELATION TO SEXUAL REPRODUCTION

While the rate of growth of the species tested was markedly affected by change in the length of the daily illumination period, the effects on blossoming and fruiting are particularly interesting and important. The experiments with soybeans included four varieties which range from early to very late in maturing under normal conditions when grown in the latitude of Washington, D. C. Thus, for plantings in the field extending through the month of May the average number of days from germination to blossoming was approximately 27, 56, 70, and 105, respectively, for the Mandarin, Peking, Tokyo, and Biloxi, the last-named showing no open blossoms till early September. Table III brings out several important facts regarding the effects of reduced light exposure on these four varieties.

TABLE III.—*Number of days required by soybeans to reach the flowering stage under daily light exposures of different lengths*

Length of daily exposure.	Mandarin.			Peking.		
	Date of germination.	Date of transfer to dark house.	Time from germination to blossoming.	Date of germination.	Date of transfer to dark house.	Time from germination to blossoming.
			Days.			*Days.*
10 a. m. to 3 p. m., 5 hours	May 17	May 20	[a] 23	May 17	May 20	[a] 23
9 a. m. to 4 p. m., 7 hours	...do....	...do....	21	...do....	...do....	21
Do	.....	.....	.....	...do....	June 7	22
Daylight to 10 a. m. and 2 p. m. till dark, 8½ to 11 hours.	.....	.....	.....	June 19	19	40
6 a. m. to 6 p. m., 12 hours	June 16	June 11	21	16	11	21
Full daylight, 12½ to 15 hours	May 17	Control	26	May 17	Control	62
Do	June 16	...do....	28	June 16	...do....	51

Length of daily exposure.	Tokyo.			Biloxi.		
	Date of germination.	Date of transfer to dark house.	Time from germination to blossoming.	Date of germination.	Date of transfer to dark house.	Time from germination to blossoming.
			Days.			*Days.*
10 a. m. to 3 p. m., 5 hours	May 17	May 20	[a] 24	May 17	May 20	[a] 27
9 a. m. to 4 p. m., 7 hours	...do....	...do....	24	...do....	...do....	26
Do	...do....	June 7	27	June 15	June 24	28
Daylight to 10 a. m. and 2 p. m. till dark, 8½ to 11 hours.	June 19	16	62	19	16	79
6 a. m. to 6 p. m., 12 hours	16	11	28	16	11	28
Full daylight, 12½ to 15 hours	May 17	Control	73	May 17	Control	110
Do	June 16	...do....	66	June 16	...do....	90

[a] In those cases in which the plants were placed in the dark house after they had germinated, only the period elapsing after they had been transferred is taken into account, rather than that beginning with the date of germination.

It is seen that when the daily illumination consists of a single exposure of 12 hours or less, the usual length of the growing period from germination to blossoming is only slightly shortened in the early variety, Mandarin; but the shortening effect is increasingly accentuated as the usual growing period increases, till, in the very late variety, Biloxi, this period is reduced to less than one-fourth that of the control plants grown under full daylight exposure during the summer months. In reality, all varieties become early maturing ones under these conditions, and there is but little difference in the time required by the four varieties to reach the blossoming stage. These tests also show that reducing the length of the illumination period below 12 hours has no further effect in shortening the vegetative period, so that apparently there is a certain minimum period of light exposure, reduction of which is without action in hastening the appearance of the flowering stage. These results seem to indicate further that for each variety a certain minimum period of time (ordinarily one of vegetative activity) must elapse from the inception of the stimulating action resulting from the reduced light exposure before the flowering stage can be attained. The data in Table III suggest

that this minimum formative period is approximately 21 days for the Mandarin and Peking varieties, 24 days for the Tokyo, and 26 days for the Biloxi, although under suitable conditions these periods might possibly be somewhat further shortened.

Subjecting the plants to two periods of illumination daily, whereby the total daily exposure averaged 9 or 10 hours, was vastly less effective in inducing early blossoming than a single daily exposure of 12 hours; and, in fact, in the later varieties the effect was of little significance. This is true in spite of the fact that the plants were in darkness during the hours of most intense sunlight—namely, from 10 a. m. to 2 p. m. Obviously it is not merely the total number of hours of sunshine received daily by the plant that may induce such marked shortening of the vegetative period, but the continuity of the exposure also plays an important part. The two plantings of soybeans serving as controls, which first appeared above ground on May 17 and June 16, respectively, did not respond in the same manner to the prevailing seasonal conditions. The vegetative period of the Mandarin was lengthened by two days as a result of the later planting, while the later maturing varieties were affected in the reverse manner. These results are in accord with the fact that the average length of day during the vegetative period was longer for the later planting than for the earlier in the case of the Mandarin, while the reverse is true of the other varieties. The marked action of a decrease in the length of the day, within certain limits, in hastening the arrival of the blossoming stage is equally in evidence throughout the stages of seed formation and maturation. This fact is shown by numerous tests; but experiments (a) (1) and (b) (3) with the Mandarin and Peking varieties, respectively, may be cited specifically.

These tests under controlled conditions clearly show that so far as concerns sexual reproduction the Mandarin soybean is adapted to a relatively long day, since the time required by it to reach the blossoming stage during the long summer days can not be greatly reduced by shortening the length of the daily exposure to light. On the other hand, the Biloxi is distinctively a "short day" variety; and with a daily light exposure of 12 hours or less it blossoms almost as early as the Mandarin, whereas the control plantings show that it refuses to blossom during the long summer days when normally exposed to the light. It is interesting to note that, on the basis of these results, all of the four varieties tested should behave similarly when grown under a 12-hour day such as prevails at the equator. The action of the shortened period of daily light exposure in promoting sexual reproduction offers a satisfactory explanation of the fact that there is a marked progressive shortening of the vegetative period in successive plantings of medium and late maturing varieties of soybeans made during the summer months. In this connection an examination of figure 1, showing graphically this progressive shortening in the vegetative period, is of interest. It should be pointed

out here that the progressive decrease in the length of the vegeta tive period of all varieties apparent in the very early plantings which germinated during the early part of May is probably due to a gradual reduction in the retarding action of relatively low temperatures which prevailed at the time. Again, there is distinct evidence of the retarding influence of lower temperatures on the very latest plantings of the Peking and Biloxi varieties. Eliminating these portions of the curves from consideration, it is evident that the graph for the early variety, Mandarin, is practically horizontal, while there is a marked downward trend in the graphs for the remaining varieties which increases in pitch as we pass toward the later varieties, the drop being quite precipitate in the curve of the very late variety, Biloxi. There is, in short, a marked tendency for the graphs to converge toward a common point as the summer season advances, a fact which is in full accord with the results of the tests under controlled conditions. Another interesting feature of these curves is that for the period around May 25 to June 15 there is a more or less well defined "hump" which is most strongly developed in the curve for the Peking, less prominent in that for the Tokyo, and hardly apparent in the curves for the Biloxi and the Mandarin. A possible explanation of this relative lengthening of the vegetative period of the Peking and Tokyo plantings which germinated during the close of May and early June is to be found in the fact that these plants received the longest possible average light exposure. This would not affect the Mandarin or the Biloxi, since the length of the day is well above the "critical" for the Biloxi and below it for the Mandarin. Apparently field plantings can not be extended through the season in such a way as to bring the plants throughout the vegetative period under a light exposure below the critical in length and at the same time secure throughout the period a sufficiently high temperature (and possibly other favorable factors) to reduce the length of the vegetative period to that which experiments conducted under controlled conditions have established as apparently the physiological minimum requisite for sexual reproduction. There can be no doubt that decreasing temperature, within limits, will retard vital activities of the plant; and the fact should be emphasized that, as a rule, the action of decreasing temperatures as fall approaches must be retarding rather than accelerating in its influence on the attainment of the flowering stage by the plant. It should be pointed out here that the hastening effect of the shorter days on the final maturation of the seed of the soybeans is shown by the fact that in the late plantings there is an evident tendency for the early Mandarin and the later Peking varieties to progress toward maturity at the same rate.

As regards the critical length of day required for furnishing the stimulus which brings into expression the processes of sexual reproduction mentioned above, it should be stated that this has not been determined as yet for any of the plants under study, and it is not possible to state how

narrowly defined this maximum length of day capable of inducing sexual reproduction may be. The outstanding fact is that it is quite different for the four varieties of soybeans. In all cases, however, it is in excess of 12 hours.

Coming to tobacco, the contrast in behavior of the Connecticut Broadleaf and the Maryland Mammoth varieties is very striking. Sexual reproduction in the Connecticut Broadleaf is not materially affected by changes in length of day within the seasonal range for the latitude of Washington or southward. On the other hand, the Maryland Mammoth, which is presumably a mutation from a very old variety of Maryland tobacco and appears to be a typical example of gigantism, can not be forced into blossoming during the summer months by any method now known except artificial shortening of the duration of the daily exposure to light, while the character of gigantism is completely suppressed when the plant is grown during the short days of winter. A glance at Table IV shows that shortening the daily light exposure has not materially affected the Connecticut Broadleaf but has been effective in shortening the vegetative period of the Maryland Mammoth. The Cuban type of Mammoth was affected like the Maryland type, but it appears that the former has a somewhat longer vegetative period than the latter under similar conditions. The Maryland type blossoms readily under the influence of a 12-hour light exposure; but there is a suggestion that a time factor is operative here, for the plants seem not to blossom so promptly as when under the 7-hour exposure. It seems probable also that the Cuban Mammoth will blossom under a 12-hour exposure to light. The observation has been made by Lodewijks (*17*) that a giant type of Sumatra tobacco—grown under the influence of the 12-hour equatorial day—which may reach the extreme height of 24 feet, either does not blossom at all or forms only a few flowers and seeds. Gigantism in tobacco disappears when the plant is brought under the influence of short days such as prevail in the temperate zone during the winter months. *Nicotiana rustica*, so far as tested, behaves like the Connecticut Broadleaf.

Aster linariifolius, again, has given clean-cut results under the different light exposures, as is shown in the summarized data of Table IV. Its behavior is strictly comparable with that of the Biloxi soybean and the giant type of tobacco. It is a typical "short-day" flowering perennial. As with the Biloxi soybean, however, this maximum length of day capable of bringing into expression the flowering and seed-formation processes is in excess of 12 hours. Exposure to light twice daily was without effect, for the vegetative period of the test plants, counting from the beginning of the experiment, was 92 days and that of the controls (not shown in Table IV) was 94 days. Here, again, attention is called to the fact that the total daily exposure to light averaged only about 10 hours, and the plants were in darkness during the period of most intense illumination, 10 a. m. to 2 p. m.

TABLE IV.—*Length of the vegetative period of tobacco and aster as affected by the length of the daily exposure to light*

Length of exposure.	Connecticut Broadleaf.		Maryland Mammoth.		Stewart 70-Leaf Cuban.		*Aster linariifolius.*	
	Date of transfer to dark house.	Length of vegetative period.	Date of transfer to dark house.	Length of vegetative period.	Date of transfer to dark house.	Length of vegetative period.	Date of transfer to dark house.	Length of vegetative period.
		Days.		*Days.*		*Days.*		*Days.*
10 a. m. to 3 p. m., 5 hours.	.....	.....	May 14	55 to 61	.....	.....	.....	.....
9 a. m. to 4 p. m., 7 hours.	June 5	43 to 49	...do....	61 to 78	.....	.....	May 13	36
Full daylight, 12 to 15 hours.	Controls	42 to 47	Controls	152 to 160	.....	.....	Controls	122
9 a. m. to 4 p. m., 7 hours.	.....	.....	May 14	52 to 72	.....	.....	.....	.....
Full daylight, 12 to 15 hours.	.....	.....	Controls[a]	140 to 164	.....	.....	.....	.....
9 a. m. to 4 p. m., 7 hours.	May 28	46 to 53	June 10	52 to 59	June 10	67 to 84	.....	.....
Full daylight, 12 to 15 hours.	Controls	36 to 44	Controls	84 to 101	Controls	81 to 90	.....	.....
Daylight to 10 a. m. and 2 p. m. till dark, 11 to 8½ hours.	.....	.....	.....	.....	.....	.....	June 16	92
6 a. m. to 6 p. m., 12 hours.	.....	.....	June 11	76 to 85	.....	.....	12	37
Full daylight, 12 to 15 hours.	.....	.....	Controls	84 to 101	.....	.....	Controls	101

[a] These controls and the test plants having a vegetative period of 52 to 72 days were in 8-inch pots.

The composite *Mikania scandens* L. is of interest as presenting a new type of plants so far as concerns behavior under long-day and short-day conditions. Under short-day conditions which were maintained for nearly 12 months this plant lost its power of blossoming. In other words, the plant became sterile. The early varieties of soybeans and the Connecticut Broadleaf tobacco blossom and fruit freely through the range of seasonal changes in the length of the day which obtains for the latitude of Washington, while the late varieties of soybeans, the giant types of tobacco, and the aster are essentially sterile when under the influence of the long summer days; and Mikania, on the other hand, is sterile during all seasons of the year except summer when long days prevail. It is worth noting that the Mikania was unable to develop flowers during the summer months when kept under the influence of a short daily exposure to light, notwithstanding that it had been growing in the greenhouse for several months previously.

The bean from the Tropics, *Phaseolus vulgaris*, included in the tests, brings us a step nearer to complete sterility in the latitude of Washington (approximately 39°), for whether it is able to blossom here will depend on the early or late occurrence of killing frost. Evidently it could not blossom very far northward of Washington. Under the influence of a 7-hour daily illumination this bean blossomed in 28 days, and one month later some of its seed pods were mature; yet under outdoor conditions

blossoming did not occur till October 11, 109 days after germination. The fact that this plant does not blossom here till the middle of October indicates that the critical length of day for flowering can not be much in excess of 12 hours; and the physiological minimum for the vegetative period appears to be approximately 28 days, about the same as for the Biloxi soybean. This bean would seem to be admirably adapted to tropical conditions.

The writers are informed by Dr. Shoemaker that in tests made by him at Washington this species in the greenhouse blossomed freely during the winter and developed seed. In the spring some of the plants, having been transferred to pots after the tops had been largely removed, were placed out of doors. New shoots developed, and these grew throughout the summer without blossoming. It is clear that this plant behaves like the Mammoth or giant type of tobacco toward differences in the length of day.

Ragweed is still another example of a short-day plant, for, under a 7-hour exposure, the anthers of the staminate heads were shedding pollen freely within 27 days after the beginning of the test, while under outdoor conditions blossoming did not occur till 7 weeks later. Radish is a good example of the type requiring a long day for attainment of the flowering stage, for, like Mikania, it has not been able to blossom under a 7-hour exposure although the test was continued throughout the summer, while under outdoor conditions blossoms appeared one month after germination. Throughout the test the rosette type of leaf development was maintained under the shortened light exposure, and both leaf and root continued to grow; so here, once more, is apparently a manifestation of gigantism. Under the conditions of the tests, the two biennials, cabbage and carrot, showed no decided response to shortened light exposure so far as concerns flowering; but their behavior under normal conditions indicates that they are to be regarded as typically long-day plants. Hibiscus is a striking example of a long-day plant, for not only is it unable to blossom under a 7-hour light exposure but it is also unable to make any appreciable growth under these conditions. The behavior of Viola is of interest because of the habit of forming both cleistogamous and chasmogamous flowers, the two types appearing at different seasons. It appears that the later developing cleistogamous flowers are to be regarded as forming the more distinctively reproductive organs. Under a 7-hour light exposure, which was not begun till June 7, the plants showed open, purple, petalliferous flowers during the first week in July, although, of course, a previous crop of these blossoms had been produced earlier in the season. The cleistogamous blossoms appeared also on the plants at the usual time, in June. The early goldenrod used in the tests showed no shortening of the vegetative period under a 7-hour exposure.

RELATIONSHIPS BETWEEN ANNUALS, BIENNIALS, AND PERENNIALS

It is well known that there are no hard and fast lines of distinction separating annuals, biennials, and perennials; for plants may change from one of these types to another under influences of environment, although in the past the particular factors of the environment involved have not for the most part been understood. The experiments recorded in this paper make it clear that in any particular region the relative lengths of the days and nights running through the year constitute one of the controlling factors in determining the behavior of plants in this particular. The soybean is commonly regarded as a typical annual in that its entire life cycle is completed in a single season, and coincident with or soon following the maturation of the seed the plant as a whole perishes. As recorded on page 567, however, a suitable change in the length of the daily exposure to light revived the vegetative life of the matured plants. After the first crop of seed had ripened and the foliage had yellowed just as usual immediately before the plant died, new shoots developed on the old stems, vegetative activity was resumed, and, finally, with the approach of the shorter days of autumn, the plants blossomed and fruited a second time. Thus, under controlled conditions the plant simulated the behavior of a flowering perennial except that the two cycles of alternate vegetative and reproductive activity have been crowded into a single season. Ragweed behaved in essentially the same manner. To make the analogy more convincing, attention is directed to the fact that aster, a flowering perennial, under the same treatment gave exactly the same results as the soybeans and ragweed. Thus, the aster readily completed two complete annual cycles within a period of about four months, except that, in the absence of low temperature, the original growth above ground, of course, was not killed. Moreover, in the second period of vegetative activity new shoots were sent up from the roots in addition to the new axillary shoots appearing on the original stems. The first flowering and fruiting of the soybeans, ragweed, and aster were forced by artificially shortening the length of the day. When the plants were restored to the full exposure of the normal summer day, vegetative activity was resumed, and, finally, the natural shortening of the days in August and September resulted in the second flowering and fruiting periods. The factor of the environment which makes the cycle of alternating vegetative and reproductive activities an annual event would thus seem to be the annual periodicity in the length of day. If temperature differences are assumed to be the primary factor, annual periodicity in tropical regions (not including the immediate vicinity of the equator) is not readily explainable.

As has already been pointed out, the Mammoth or giant type of tobacco behaves as a typical flowering annual, like the ordinary tobaccos, when grown under the influence of days not exceeding 12 hours in length. During the winter months the plant blossoms readily and, in fact, becomes practically an ever-blooming type. It is an interesting fact, however,

that as the seed capsules mature the seed-bearing stem dies back only to the first node which may have sent up a new branch. This holds true even though the new branch be but a few inches below the seed head. The portion of the stem below the new branch and the root system henceforth function as parts of a new plant. In winter the new branch blossoms and fruits promptly, perishes, and is succeeded by new branches. As spring advances the new branches coming out assume the giant or nonflowering type of growth which continues till fall brings a return of the short days, when blossoms promptly appear. It would seem that the new branch acts as a rejuvenating or a protective agent against the death of the older organs to which it is attached. Obviously the Mammoth tobacco resembles both the annual and the perennial types of plant life. The sharpness with which the new branch controls the extent of the dying-back of the mother stem is shown in Plate 76, A.

In the latitude of Washington the radish is an annual unless planted very late in the season. It has already been shown that under a shortened light exposure, on the other hand, while vegetative development may continue, flowering does not occur. It would appear from this that the radish might not flower in regions where the maximum length of days is relatively short; and, in fact, according to Dr. Walter Van Fleet, of the Bureau of Plant Industry, the radish as a rule does not blossom when grown in the equatorial region. Similarly, the radish blossoms only occasionally as far north as Porto Rico, where the principal growing season is during the winter months (*13*). This behavior of the radish, again, is obviously an approach toward the nonflowering type of perennial. Similarly, Dr. Van Fleet states that a lima bean coming under his observation in the Tropics had continued to grow as a perennial for a number of years, having attained giant proportions, while there was only occasional and sparse fruiting. Conversely, the beet ordinarily is a biennial in the latitude of Washington, but when grown in Alaska where the summer days are very long, it is likely to develop seed and thus complete its life cycle in a single season. The intimate relationship existing between the length of day and the attainment of the reproductive stage is strikingly shown by the behavior of the radish under special conditions. In the box of plants used as controls in the experiment described on page 565 and discussed above, the great majority of the individuals developed normal flowering stalks and seed pods in due season (see Pl. 75, B). A few individuals, however, developed considerably later, because of delayed germination or some other reason; and these delayed plants began the formation of flowering stalks. The length of the day having decreased to the critical length, the growth of the seed stalk was arrested after a height of a few inches was attained; and instead of the normal flower head, a crown of foliage leaves developed, as shown in Plate 69, B, thus indicating the resumption of vegetative activity. What is believed to be another example of the directing

action of relative day length is the behavior of certain northern varieties of pepper (Capsicum) when planted in Porto Rico in the spring months (*13*). Under these conditions the peppers imported from the higher latitudes of the United States were able to form only a very few fruits before they began to yellow and shed their foliage, after which the plants soon perished. Also, it is stated that the radish when grown in Porto Rico during the winter months behaves as it does when grown nearer the equator. The above-mentioned experimental results and observations seem to justify the conclusion that the relative length of the day through the year is a factor of the first importance in determining whether many plants behave as annuals, biennials, or perennials, and whether reproduction in such plants is vegetative or sexual or both in any particular region.

The forcing of two flowering periods in a single season under controlled conditions naturally directs attention to another phase of periodicity in plant activity—namely, the appearance of the blossoming period in both spring and fall, or only in one of these seasons in regions outside the Tropics. This question is of special interest with respect to perennials. It is apparent that plants blooming only in the spring or fall or in both seasons are to be regarded as requiring relatively short days for attaining this stage. In annuals, ordinarily a period of vegetative development must necessarily precede flowering, so that the latter stage is likely to be deferred till autumn; but when propagation is by means of bulbs or other reproductive storage organs, blossoming may well occur in the spring. In hardy shrubs and trees a typical condition is that in which the formation of flowers or flower buds is inaugurated in the autumn under the influence of the shortening days, while the flowering process is interrupted before completion through the intervention of cold weather. The result is that actual blossoming usually takes place in the spring; but if the fall or early winter temperatures are abnormally high, the flowering process may be completed before cold weather intervenes. This phenomenon is occasionally observed in the apple. In the spring, temperature would be the chief factor in determining the date of blossoming for this class of plants. It is suggested that the seasonal distribution of flower-bud formation in the lemon which is considered in a recent interesting article by Reed (*22*) may be due to these light and temperature relations. The process is most active during the late fall and again in very early spring, with a winter period of low activity. Throughout the summer period of long days, also, activity is at a minimum.

LENGTH OF DAY CONTRASTED WITH LIGHT INTENSITY

As early as 1735 Reaumer (*21*) undertook to make accurate comparisons of the total quantities of heat required to bring plants to given stages of maturity. At intervals since that time this idea has been

revived, and serious efforts have been made to establish some form of quantitative relationship between plant development and the quantity of heat received from the sun. The work of Linsser (*15*, *16*) and of Hoffman (*11*, *12*) in this field is worthy of special mention. In this connection, also, Abbe's critical review of investigations having to do with the relations between climates and crops is of interest (*1*). It is believed that the results of the present investigation have an important bearing on the subject. Since the quantity of solar radiation received directly by the plant is the product of the intensity and the length of the exposure, it might be expected that any relationship existing between plant processes and the total quantity of radiation received would be disturbed by changes in either the intensity of the light or the duration of the exposure to its action. It has been shown that the relative length of the day is a factor of the greatest importance in relation to reproductive processes in the plant, and it will be of interest to consider whether the intensity of the solar radiation is also of special significance. At the outset it may be observed that it hardly seems likely that light intensity could exert a controlling influence on reproduction in plants, in view of the extent to which the response of plants to differences in light intensity has been studied by investigators without discovery of any very significant relationships so far as concerns reproduction. In the experiments discussed in preceding paragraphs it was found that where daily exposures of 7 hours and 12 hours, respectively, were equally effective in shortening the vegetative period, a total daily illumination aggregating on an average 9 to 10 hours but consisting of two separate exposures, with a 4-hour period of darkness intervening, was vastly less effective in this respect. This shows at once that the total quantity of radiation received can not be responsible for the shortening of the vegetative period produced by shortening the single daily exposure to light. Furthermore, since in the double daily exposures the intervening period of darkness to which the plants were subjected, 10 a. m. to 2 p. m., was at the time of day when the intensity of the solar radiation reaching the earth's surface is at its maximum, the average intensity of the radiation received by these plants is less than that received by those plants which were exposed continuously from 9 a. m. to 4 p. m.

The Stewart Cuban Mammoth tobacco which requires a day length of 12 hours or less to attain the blossoming stage has been grown commercially to some extent under an artificial shade of coarse cheesecloth estimated to reduce the intensity of the sunlight by approximately one-third. It has been observed that this shade has had no noticable effect on the date of blossoming of the tobacco. Again, the aster used in the present investigation grows in the wild state under a variety of situations, some of which are very shaded, but observation during the past

season showed that there was no appreciable difference in dates of flowering under these varied exposures.

Further evidence on this subject is furnished by the following experiments in which soybeans were subjected to different degrees of shading, primarily for determining the effect on oil formation in the seed. Different types of shade were employed, and in some instances shading was combined with regulated differences in the water supply of the soil. In all these experiments the aim has been to use a type of shade which would reduce to a minimum secondary effects, such as modifying the air temperature and the temperature and moisture content of the soil. The object, in short, was to measure, as far as practicable, only the direct action of different light intensities on the plant itself, though, of course, this goal can not be fully attained. With this aim in mind the triangular type of shade, shown in Plate 76, B, was used in a series of tests made in 1916. For this shade the standard cheesecloth of best grade, extensively used for surgical dressings, was employed (see Pl. 77, E). The opening extending around the shade near the top, with loose overhanging flap, is for the purpose of facilitating ventilation. The arrangement is such that the frame of the shade can be raised from time to time to accommodate the growth of the plants. The width of the frame was 4 inches at the base and 18 inches at the top, and it was 30 inches high. In these as in the later tests the Peking variety of soybean was used. It will be recalled that this variety is quite sensitive to changes in the length of the day.

The simplest and perhaps the most satisfactory type of shade was that employed in 1917 and 1918. A frame of iron pipe, 30 inches high, 40 inches wide, and of the desired length, was used to support the cloth. The shades in all cases extended almost due east and west. The beans in each instance were planted in a row 6 inches to the north of the center line of the shade to allow for the southerly swing of the sun's course through the sky. Comparatively open, loosely woven cloth, of the type used for the commercial culture of cigar-wrapper tobacco in New England and Florida, was used for this shade. Four different weaves of cloth were used—6 by 6, 8 by 10, 12 by 12, and 12 by 20 mesh, these figures indicating the average number of threads to the linear inch. These cloths are shown in natural size in Plate 77, A–D.

In 1918 tests were extended to include differences in water supply in combination with three different degrees of shading (see Pl. 78, A). This was accomplished by planting the beans in wooden boxes 24 feet long, 12 inches wide, and 14 inches deep, each dox being divided by partitions into three 8-foot sections. These boxes were set in the soil so as to extend about 2 inches above the surface and were filled with soil up to 2 inches of the top. Under each degree of shading, three different soil-moisture contents were maintained, designated as wet,

medium, and dry. Rainfall was largely excluded by laying boards over the boxes on each side of the plants, the boards having sufficient pitch outward to turn the flow of the water. In addition, control plantings were made in the field, a portion without shade and the remainder covered with the shade cloths; and these received no water except the rainfall. Only those features of the test which relate to shading will be considered here, details of the differences in water supply and their effects pertaining more properly to the next section of the paper. To ascertain whether the simplified form of shade exerted any decided indirect effect through the soil, soil thermographs were installed in the soil at a depth of 3 inches under the 12 by 20 cloth shade, in a position near the plants and in a similar position on the field row receiving no special treatment. No significant differences in the temperature records were obtained.

A matter of special importance, of course, is the degree of shading produced by the different types of shade and different weaves of cloth used. For several reasons only approximations can be had as to the intensity of the light received by the plants under the shades. The positions of different plants and different parts of the same plant with respect to the light necessarily vary, and the shape of the shade involves a constantly changing transmission rate by the shade cloth. The normal daily range in light intensity is magnified by the shade, since the coefficient of transmission of the cloth is greatest at midday and decreases toward sunrise and sunset. In the 1916 type of shade there is a relatively small coefficient of light transmission furnished by the sloping side walls covered with cheesecloth. In the simplified type of shade only the transmission through the top comes into consideration, since there are no side walls. The southward extension of the top is such, however, that only diffuse light reaches the plants from the side, with the exception of their extreme lower portions, which are exposed to the direct sunlight in the early morning and late afternoon. In the open type of shade, diffuse light naturally becomes a larger factor. Observations made by Prof. H. H. Kimball, of the United States Weather Bureau, by means of the pyrheliometer gave transmission coefficients of 0.441, 0.292, 0.452, 0.613, and 0.727, respectively, for cheesecloth 12 by 20, 12 by 12, and 8 by 10, and for 6 by 6 mesh netting when exposed normally to the sun's rays. Formulas also were developed by Prof. Kimball which make it possible to compute the shading effect at any hour of the day and for any date. Since the sun's rays never strike the shade cloth at normal incidence, the maximum intensity of the transmitted light, which is attained at midday, is slightly less than indicated by the above values. The computed shading effect produced by each type of netting at various hours of the day on June 1, July 1, and August 1 is shown in Table V. It is seen that for horizontal exposures the shading effect is almost constant from 10 a. m. to 2 p. m. but increases considerably from 10

a. m. to 8 a. m. and from 2 p. m. to 4 p. m. and increases very rapidly from 8 a. m. to 6 a. m. and from 4 p. m. to 6 p. m. For vertical exposures the reverse relations, of course, obtain.

TABLE V.—*Computed shading effect of netting of various weaves and of cheesecloth at different hours of the day during the summer months, with horizontal exposure of the netting and cheesecloth and also with vertical exposure of the cheesecloth*

[Complete shading represented by unity]

Kind of material.	June 1.				July 1.				August 1.			
	Noon.	10 a. m. and 2 p. m.	8 a. m. and 4 p. m.	6 a. m. and 6 p. m.	Noon.	10 a. m. and 2 p. m.	8 a. m. and 4 p. m.	6 a. m. and 5 p. m.	Noon.	10 a. m. and 2 p. m.	8 a. m. and 4 p. m.	6 a. m. and 6 p. m.
6 by 6 netting..	0.30	0.30	0.37	0.66	0.29	0.31	0.36	0.61	0.30	0.31	0.37	0.69
8 by 10 netting.	.41	.43	.51	.90	.41	.42	.50	.83	.41	.43	.51	.94
12 by 12 netting.	.56	.58	.69	1.0	.55	.58	.78	1.0	.56	.59	.69	1.0
12 by 20 netting.	.69	.71	.83	1.0	.68	.71	.81	1.0	.69	.72	.84	1.0
Cheesecloth (top).....	.57	.59	.70	1.0	.57	.59	.69	1.0	.56	.59	.71	1.0
Cheesecloth (vertical sides).	1.0	.78	.62	.57	1.0	.81	.63	.57	.96	.77	.62	.57

To obtain further information as to the shading effect of the nettings used, a section of the simplified type of shade, without side covering, was set up and covered with the 12 by 12 netting. Under this shade (about 6 inches below the netting) Livingstone standardized black and white spherical atmometer cups were installed, and corresponding control cups were placed in full sunlight in the open air. In general, it was found that satisfactory results could not be secured when the wind was blowing; but when there was no appreciable breeze, readings were obtained which seemed to indicate a coefficient of light transmission reasonably close to that determined by Prof. Kimball. Typical readings obtained on clear, calm days are given in Table VI.

TABLE VI.—*Readings of black and white spherical atmometer cups under 12 by 12 netting and in direct sunlight, and the indicated coefficient of light transmission, 1919*

Date.	Period of exposure.	Readings.				Difference.		Indicated coefficient of light transmission for 12 by 12 net.
		Under the net.		In the open.		Under the net.	In the open.	
		Black cup.	White cup.	Black cup.	White cup.			
		Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	
Aug. 26	10.45 a. m. to 11.45 a. m.	4.8	3.5	6.0	3.7	1.3	2.3	0.56
26	10.45 a. m. to 3 p. m.....	24.3	18.0	29.6	19.5	6.3	10.1	.62
28	9 a. m. to 3 p. m.....	20.7	14.4	26.9	15.8	6.3	11.1	.56
29	10.15 a. m. to 3 p. m.....	18.2	12.8	22.4	13.0	5.4	9.4	.57

In the 1916 experiments the soybeans were planted June 21, and the shade was placed in position July 5. Detailed observations were made on the growth and development of the shaded plants and of the unshaded controls. There were 93 individuals under the shade and 67 in the

control row. The summarized data in Table VII will bring out the comparative behavior of the shaded and unshaded plants.

TABLE VII.—*Effect of shading soybeans with cheesecloth, 1916*

Treatment.	Average height.	Air-dry weight per stalk, defoliated.	Yield of beans per stalk.	Yield of hulls per stalk.	Percentage of beans in seed pods.	Date of blossoming.
		Gr.	*Gr.*	*Gr.*		
Plants shaded	3 ft. 5 in.	5.4	10.5	5.4	66.1	Aug. 7
Plants not shaded	2 ft. 3 in.	9.9	17.0	9.0	65.2	Do.

The shaded plants show the typical effects of reduced light intensity so often observed—increased elongation of stem, slender growth, enlarged area of leaves, reduced production of dry matter. Besides these effects the yield of seed was considerably reduced. For present purposes the important fact is that although the maximum intensity of the direct light reaching these plants was only about 43 per cent of the normal, the date of blossoming was not affected in the slightest degree. This is a striking contrast with the fact that by reducing the length of the daily light exposure from an average of approximately 14 hours to 12 hours, or about 15 per cent, the length of the period from germination till blossoming was reduced from 51 to 21 days. It was observed, however, that the seeds of the shaded plants were about a week later than those of the control plants in reaching final maturity.

In the 1917 tests the beans were planted June 27 and the shades placed in position a few days after germination for the first series, while in a second series the shades were set up at the time of blossoming. Two grades of netting were used, the 6 by 6 and the 8 by 10 mesh. The general behavior of the plants is shown in Table VIII. Here, again, it is seen that reducing the intensity of the direct sunlight to maxima of about 70 and 59 per cent, respectively, of the normal has shown no effect on the date of flowering.

TABLE VIII.—*Effect of shading soybeans with 6 by 6 and 8 by 10 mesh cotton netting, 1917*

Treatment.	Number of individuals grown.	Average height.	Air-dry weight per stalk, defoliated.	Yield of beans per stalk.	Yield of hulls per stalk.	Percentage of beans in seed pods.	Date of blossoming.
		Inches.	*Gr.*	*Gr.*	*Gr.*	*Gr.*	
6 by 6 netting from germination to maturity	72	28	4.7	9.4	5.1	64.6	Aug. 17
6 by 6 netting from blossoming to maturity	67	24	5.8	9.9	5.0	66.8	Do.
8 by 10 netting from germination to maturity	75	25	3.7	7.0	4.1	62.9	Do.
8 by 10 netting from blossoming to maturity	65	25	5.4	9.4	5.4	63.4	Do.
Not shaded	305	22	4.8	8.5	5.1	62.7	Do.

TABLE IX.—*Effect of various degrees of shading in combination with differences in water supply on the growth and development of soybeans, 1918*

Treatment.			Number of plants grown.	Average height.	Weight per stalk, defoliated.	Weight of hulls per plant.	Weight of beans per plant.	Percentage of beans in seed pods.
Shade.	Moisture.	Duration.						
				Inches.	*Gr.*	*Gr.*	*Gr.*	
12 by 12 netting	Wet	Germination till maturity.	40	31	9.8	8.4	14.5	63.5
Do	Medium	do	42	26	6.1	5.6	10.2	64.5
Do	Dry	do	44	21	3.4	3.5	6.8	65.8
6 by 6 netting	Wet	do	43	31	9.6	8.5	14.3	62.8
Do	Medium	do	47	28	6.7	6.7	11.4	63.0
Do	Dry	do	48	21	3.1	3.1	6.9	69.4
12 by 12 netting	Actual rainfall.	do	63	31	9.4	8.1	15.7	66.1
6 by 6 netting	do	do	58	31	9.6	8.6	16.0	65.6
12 by 20 netting	do	do	47	34	9.3	7.5	15.0	66.9
Not shaded	do	do	110	26	9.9	8.5	16.4	65.7
Not shaded[a]	do	do	77	31	13.5	10.5	24.8	70.2
12 by 12 netting	Wet	Blossoming till maturity.	43	28	7.7	6.3	14.5	69.9
Do	Medium	do	45	30	7.8	7.1	13.1	65.0
Do	Dry	do	45	29	7.0	4.8	11.0	69.5
6 by 6 netting	Wet	do	45	32	8.7	6.7	13.3	66.3
Do	Medium	do	44	31	8.3	6.5	12.7	66.3
Do	Dry	do	44	25	7.3	6.5	11.6	63.9
Not shaded	Wet	do	46	31	7.8	7.2	13.1	64.9
Do	Medium	do	48	32	7.9	6.3	12.3	66.2
Do	Dry	do	48	24	5.7	4.9	8.8	64.3
12 by 12 netting	Actual rainfall.	do	58	30	10.1	8.2	16.8	67.3
6 by 6 netting	do	do	62	30	10.6	9.8	17.7	64.4
12 by 20 netting	do	do	49	32	9.7	8.2	16.9	67.2

[a] This planting differed from the control immediately preceding only in that the plants were spaced 5 to 6 inches apart in the row while in all other cases they were spaced 2 to 3 inches apart.

In the 1918 experiments the plantings were made from June 4 to 6. Two different degrees of shading were used in combination with three different rates of water supply in each of two series, one covering the period from germination to maturation and the other extending only from blossoming till maturation. In addition, two corresponding series were run, in each of which three different degrees of shading were employed without variation in the water supply, the plants in this case being grown in open field rows without use of boxes, so that the actual rainfall of the season was received by the soil. As controls, a series was arranged without shade but with the three rates of water supply, which extended only from the blossoming period till maturation, the plantings being in buried boxes as in the other experiments having to do with water supply. An additional control consisted of a planting in the field without any special treatment as to either shade or water supply; and, incidentally, a similar planting was made which differed only in that the plants were spaced 5 to 6 inches apart instead of the standard distance of 2 to 3 inches used in all other cases. The shades for the two periods of shading were placed in position, and the special water treatments were begun on June 12 and 13 and August 9, respectively. The results of the tests are summarized in Table IX. It appears that the effect of the shade on the size, weight, and relative proportions of the plant parts is

dependent to a considerable extent on the relative water supply. In general, however, reduction in light intensity during the period from germination till maturity gives results similar to those obtained in the preceding tests; and there is a tendency toward a reduced yield of seed, as previously noted. Reducing the intensity of light during the period between blossoming and final maturity, on the other hand, appears to increase somewhat the yield of seed. Without exception, the plants began blossoming on August 7 under all treatments as to shade and differences in water supply, applied either singly or in combination. In these tests it is estimated that under the heaviest shading the maximum intensity of the direct sunlight reaching the plant was only 32 per cent of the normal, and the average for the day could scarcely have exceeded 25 per cent of the normal.

RELATION OF OTHER FACTORS OF THE ENVIRONMENT TO REPRODUCTION

Having seen that under the conditions of the experiments described in the previous section differences in light intensity were without effect on the length of the vegetative period which preceeds flowering in soybeans, it is worth while considering whether other factors of the environment, especially water supply and temperature, are of significance. In studying the relation of the water supply to the formation of oil in the seed, a number of tests have been made with soybeans, beginning with 1912; but it will suffice to consider here only the results obtained for the years 1916 and 1918 with the Peking variety. In 1916 plantings were made in a series of four boxes set in the soil and provided with board covers, just as has been described in the preceding section (see p. 583). Each of the boxes was 12 feet long, 12 inches wide, and 12 inches deep. In one of these boxes the soil was maintained in a relatively moist condition from germination to final maturity, and in a second one the soil was kept comparatively dry during this period. In the third box the soil moisture was kept the same as that in the first box till the most active flowering stage was past, after which the moisture content was reduced to that of the second box. In the fourth box the soil was kept relatively dry till the flowering stage was past and thereafter in a relatively moist condition. A control planting receiving the actual rainfall was also made in the field. The beans were planted June 21, and the addition of water to the boxes began July 17. The transition in the moisture relations of the third and fourth boxes was begun August 19. The appearance of the plants in the boxes in the late summer is shown in Plate 79, A. The quantities of water supplied to the boxes each week, together with the rainfall for the period of the tests, are given in Table X. Determinations of the moisture content of the soil in the boxes were made at intervals through the month of August. Experience has shown that in the field the soil used in these tests contains 16 to 18 per cent moisture when in best condition

for most crop plants. The results of the moisture determinations in the boxes are shown in Table XI.

TABLE X.—*Quantities of water added to boxes and the rainfall during the period of the tests dealing with effect of differences in soil moisture on the development of soybeans, 1916*

Week ending—	Box 1, wet from germination to maturity.	Box 2, dry from germination to maturity.	Box 3, wet from germination to blossoming; dry thereafter.	Box 4, dry from germination to maturity; wet thereafter.	Rainfall (on field planting only).
	Gallons.	*Gallons.*	*Gallons.*	*Gallons.*	*Inches.*
July 24	32	0	32	0	1.77
31	20	0	20	0	2.14
Aug. 7	20	0	20	0	.56
14	18	4	18	4	.23
21	18	4	4	12	.38
28	20	2	8	12	.92
Sept. 4	12	4	6	6	.03
11	37	6	6	26	.70
Total	177	20	114	60	6.73
Equivalent to	[a] 23.6	[a] 2.7	[a] 15.2	[a] 8	.....

[a] Inches.

TABLE XI.—*Moisture content of soil in boxes used for growing soybeans, 1916*

Date of examination.	Box 1, wet from germination to maturity.	Box 2, dry from germination to maturity.	Box 3, wet from germination to blossoming; dry thereafter.	Box 4, dry from germination to maturity; wet thereafter.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
July 17	13.0	13.0	13.0	13.0
Aug. 1	20.6	11.0	21.2	11.0
8	20.6	11.0	13.8	11.0
15	15.5	9.5	15.5	9.5
22	14.7	10.0	10.8	16.8

The comparative growth and development of the plants under the different treatments are indicated by the data presented in Table XII. It appears that the control plants in the field were somewhat larger and considerably more productive than the best plants in the boxes, which were those receiving the larger water supply from germination to maturity. These differences were possibly due to the larger volume of soil available to the plants in the field. There are large reductions in the size and productiveness of the plants in the boxes resulting from a deficiency in the water supply. It appears also that a more favorable water supply during the period preceding the flowering stage resulted in greater vegetative development, while a more favorable water supply after the flowering stage gave a larger yield of seed. In spite of the well-defined

differences in the size of the plants and their fruitfulness brought about by differences in the water supply, the date of blossoming was not affected at all, the first blossoms in all boxes and in the field appearing August 7. No important differences were observed in the time of final maturation under the different treatments.

TABLE XII.—*Effect of differences in the moisture content of the soil on the growth and development of soybeans, 1916*

Treatment.	Number of plants grown.	Average height.	Air-dry weight per stalk, defoliated.	Weight of seed hulls per stalk.	Weight of beans per stalk.	Percentage of beans in seed pods.
		Inches.	*Gr.*	*Gr.*	*Gr.*	
Soil wet from germination to maturity.....	64	27	8.0	7.3	12.4	63.0
Soil dry from germination to maturity.....	67	19	4.1	3.1	8.1	62.4
Soil wet from germination to blossoming and dry thereafter.....	69	26	7.4	3.2	8.6	62.2
Soil dry from germination to blossoming and wet thereafter.....	57	21	5.5	6.6	11.5	63.5
Control, in field under actual rainfall.....	67	27	9.8	9.1	17.0	65.2

It may be observed in passing that the wooden boxes placed in the soil as indicated above have been found to be very satisfactory for conducting field tests dealing with the effects of the water supply on plants. By the arrangement of sloping covers on either side of the plants rainfall can be very largely excluded, and losses of soil moisture from causes other than transpiration are reduced to a minimum. Boxes of any convenient size and length may be used, and placing the boxes in the soil insures a close approach to general field conditions.

The general plan as well as a summary of the results of the 1918 tests on the effects of differences in water supply in combination with different degrees of shading have been given in the preceding section on light intensity. It is appropriate to give here further details of the water treatments. The surface area of the soil in each 8-foot section of the boxes was 8 square feet, so that nearly 5 gallons of water would be required to supply the equivalent of a rainfall of 1 inch. The water was applied in measured quantities by means of a garden hose. Although nearly all water lost by the soil was through transpiration, it was found necessary to water heavily each day in periods of hot and dry weather. The quantities of water added weekly and the rainfall during the period of the tests are shown in Table XIII.

Soil moisture determinations were made at intervals during the season from samples taken from the field and from the boxes. The samples were taken to a depth of 12 inches—that is, to the bottom of the soil in the boxes. Composite samples were made up from boxes receiving the same quantities of water. The samples were taken in all cases just before

adding water to the soil, so that the average moisture contents would be somewhat higher than these figures. Results of the moisture determinations are shown in Table XIV.

TABLE XIII.—*Quantities of water added weekly to soybeans, and the rainfall during the period of the tests dealing with effect of differences in soil moisture, 1918*

Week ending—	From germination to maturity.						From blossoming to maturity.									Rainfall on controls.
	12 by 12 net shade.			6 by 6 net shade.			12 by 12 net shade.			6 by 6 net shade.			Not shaded.			
	Wet.	Medium.	Dry.	Wet.	Medium.	Dry.	Wet.	Medium.	Dry.	Wet.	Medium.	Dry.	Wet.	Medium.	Dry.	
	Gall.	*Gall.*	*Gall.*	*Gall.*	*Gall.*	*Gall.*	*Gall.*	*Gall.*	*Gall.*	*Gall.*	*Gall.*	*Gall.*	*Gall.*	*Gall.*	*Gall.*	*In.*
June 21	0.5	0.5	(a)	0.5	0.5	(a)	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	0.11
28	4.0	1.0	(a)	4.0	1.0	(a)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	.87
July 5	9.0	7.0	(a)	9.0	7.0	(a)	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	.58
12	3.0	2.0	1.5	3.0	2.0	1.5	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	.57
19	3.0	2.0	1.0	3.0	2.0	1.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	1.41
26	6.0	6.0	6.5	6.0	6.0	6.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	.71
Aug. 2	8.0	8.0	3.0	8.0	8.0	3.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	.69
9	24.0	18.0	9.0	24.0	18.0	9.0	22.0	20.0	18.0	22.0	20.0	18.0	22.0	20.0	18.0	.46
16	24.0	22.0	5.0	24.0	22.0	5.0	24.0	22.0	4.0	25.0	22.0	4.0	24.0	22.0	4.0	1.03
23	24.0	22.0	5.5	24.0	22.0	5.5	24.0	22.0	5.5	24.0	22.0	5.5	24.0	22.0	5.5	.26
30	30.0	20.0	8.0	30.0	20.0	8.0	30.0	20.0	8.0	30.0	20.0	8.0	30.0	20.0	8.0	.44
Sept. 6	28.0	18.0	6.0	28.0	18.0	6.0	28.0	18.0	6.0	28.0	18.0	6.0	28.0	18.0	6.0	.44
13	8.0	6.0	2.0	8.0	6.0	2.0	8.0	6.0	2.0	8.0	6.0	2.0	8.0	6.0	2.0	.51
20	18.5	14.0	5.5	18.5	14.0	5.5	18.5	14.0	5.5	18.5	14.0	5.5	18.5	14.0	5.5	1.77
27	8.0	4.0	4.0	8.0	4.0	4.0	8.0	4.0	4.0	8.0	4.0	4.0	8.0	4.0	4.0	.62
Total	198.0	150.5	57.0	198.0	150.5	57.0	196.5	160.0	87.0	196.5	160.0	87.0	196.5	160.0	87.0	10.47
Equivalent to	b 39.6	b 30.1	b 11.4	b 39.6	b 30.1	b 11.4	b 39.3	b 33.3	b 17.4	b 39.3	b 33.3	b 17.4	b 39.3	b 33.3	b 17.4	.....
For period from Aug. 2 to Sept. 27							32.5	52.2	10.6	32.5	25.2	10.6	32.5	25.2	10.6	5.5

a None. b Inches.

TABLE XIV.—*Water content of soil in boxes and in the field during the period of the tests with soybeans, 1918*

Date of sampling.	From germination to maturity.			From blossoming to maturity.						In field.
	12 by 12 and 6 by 6 netting.			12 by 12 and 6 by 6 netting.			No shade.			
	Wet.	Medium.	Dry.	Wet.	Medium.	Dry.	Wet.	Medium.	Dry.	
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
June 19	19.4	18.6	17.1	.....	.....	.....	.....	.....	.....	.....
July 1	19.5	16.4	15.3	20.6	20.6	20.6	20.6	20.6	20.6	.....
8	20.8	16.4	12.9	19.1	19.1	19.1	19.1	19.1	19.1	.....
15	21.9	18.0	14.6	18.8	18.8	18.8	20.2	20.2	20.2	.....
23	16.7	13.1	11.3	18.1	18.1	18.1	18.3	18.3	18.3	.....
Aug. 2	17.1	12.5	10.4	16.6	16.6	16.6	15.5	15.5	15.5	.....
13	.....	.....	10.3	.....	.....	10.3	.....	.....	.....	10.8
16	19.6	18.2	12.2	19.6	18.2	12.2	19.6	18.2	12.2	11.9
20	.....	.....	8.4	.....	.....	10.0	.....	.....	11.0	11.5
Sept. 5	22.1	18.7	11.5	22.1	18.7	11.5	22.1	18.7	11.5	8.3

The rainfall was relatively light but extraordinarily uniform in distribution during the season; and, though the field soil was comparatively dry most of the time, the plants did not show wilting at any stage. In the boxes the plants in the "dry" sections were kept in a condition in which wilting frequently occurred in the middle of the day through the season. The boxes were 12 inches in width while the distance between field rows was 3.3 feet. It is interesting to note, therefore, that the plants in the boxes, which had slightly less than a third as much lateral soil area from which to draw their moisture as was available to the plants in the field, required an addition of water equal to three times the rainfall in order to attain the same development as was reached by the field plants. When this condition was attained—that is, in the "wet" boxes—the size of the plants was almost exactly the same as that of the plants in the field.

It is seen at once (Table X) that in the boxes the water supply is the chief limiting factor, for the height of the plants, the size of the stalks, the production of seed, etc., are greatly affected by the quantity of the water supplied. On the other hand, reduction of the water supply, even to the point where almost daily wilting of the plants occurred, did not change the time of flowering by a single day. Changing the water supply after the flowering stage likewise produced decided effects on the further development of the plants, and in the same general direction as noted above, although naturally the changes are not so great as when the differences in water supply are maintained throughout the active period of the plant's life. As regards maturation, it was observed that the plants in the wetter soil of the boxes were perhaps a week later in shedding their leaves and ripening their seed pods than those in the field and in the drier soil of the boxes.

While water supply is the chief factor influencing plant development in the boxes, these tests furnish a clear case of the simultaneous action of two limiting factors, for the different degrees of shading likewise affected the development of the plants. Quantitatively, these two limiting factors are of decidedly unequal significance. Within the limits covered by the tests, the effects of the differences in water supply could be demonstrated in nearly all cases even if the light intensity were an uncontrolled variable. On the other hand, the effects of the differences in light intensity would be completely masked in most instances if the water supply were not rigidly controlled. This experiment illustrates the problems of soil productiveness and crop yields which confront the agronomist and clearly points to the futility of attempting to deal with limiting factors of relatively small significance, such as comparatively narrow distinctions in fertilizer requirements of given soils or crops or in the crop-yielding powers of different strains or varieties of plants.

That temperature is a factor of first importance in influencing and controlling plant activities is well understood, and it needs to be considered here only in its relations to the length of day as a factor playing a dominant rôle in the reproductive processes of the plant. It is well known that in accordance with Vant Hoff's law the speed of chemical reactions is doubled for each increase of 5° to 10° in temperature; and similarly plant activities and processes such as respiration and growth are accelerated by increase in temperature, provided the optimum is not exceeded. Conversely, decrease in temperature may moderately retard the plant's activities, or this effect may increase to more or less complete inhibition. Extremes in either direction, of course, may result in killing the flowering buds or fruits, the vegetative shoots, or the entire plant. It is a matter of common knowledge that low temperature retards the development and the unfolding of flowers. An interesting interrelationship of this action of temperature and that of the seasonal decrease in length of day is seen in the behavior of such trees as the apple, previously referred to. Under the influence of the relatively short days fruit trees of this type might be expected to unfold their flowers regularly in the fall instead of in the spring were it not for the interference of low temperatures. The low temperature of winter would seem to have the effect of changing what would otherwise be among the latest flowering plants of the fall into the early flowering ones of spring.

For the temperate and frigid zones the results of the present investigation have made it clear that in some species, at least, distinctions in the time of flowering and fruiting of different varieties, which may be classed as early maturing, late maturing, nonmaturing, and sterile or nonflowering, are due primarily to responses to different day lengths which come into play as the season advances. Here, again, low temperature becomes a factor of increasing importance as the season advances, and, so far as concerns "short-day" plants, it controls the situation with respect to the conditions of nonripening of fruit and of nonflowering. With increasing latitude this relationship between the opposed action of the length of day and the falling temperature becomes more critical for the later maturing varieties. With decreasing latitude a condition is reached in the subtropics which is much more favorable to late maturing or short-day varieties, for the length of day may fall below the critical maximum for flowering without the inhibiting or destructive action of low temperatures coming into play to prevent successful fruiting. At the equator, annual periodicity of both temperature and length of day cease to play an important rôle in plant processes.

LENGTH OF DAY AS A FACTOR IN THE NATURAL DISTRIBUTION OF PLANTS

In an intelligent understanding of the natural distribution of plants over a particular area those factors which are favorable or unfavorable to growth and successful reproduction for each species must be given

consideration. Heretofore temperature, water, and light intensity relations have been considered the chief external limiting factors governing the distribution or range of plants. In the light of the observations and experimental results presented in this paper it seems probable that an additional factor, the relative length of the days and nights during the growing period, must also be recognized as among those causes underlying the northward or southward distribution of plants.[1] It is evident that the equatorial regions of the earth alone enjoy equal days and nights throughout the entire year. Provided the water relations are favorable, the warm temperatures in these regions favor a continuous growing season for plants. Passing northward from the equatorial regions into higher latitudes, temperatures promoting active vegetative growth and development are restricted to a summer period which, other conditions being equal, becomes progressively shorter as the polar regions are approached. Coincident with these changes from lower to higher latitudes, the summers are characterized by lengthening periods of daylight and the winters by decreasing periods of daylight. We may now consider how these different day and night relations operating during the summer growing period will exercise more or less control upon the northward or southward distribution of certain plants.

It is evident that a plant can not persist in a given region or extend its range in any direction unless it finds conditions not only favorable for vegetative activity but also for some form of successful reproduction. For present purposes only sexual or seed reproduction need be considered. The experiments above described have indicated that for certain plants—for example, ragweed and the aster—the reproductive or flowering phase of development in some way depends upon a stimulus afforded by the shortening of the days and the consequent lengthening of the nights as the summer solstice is passed. It remains to consider more specifically the bearing these facts may have when plants characterized by this type of behavior are subjected to the daylight relations of different latitudes. In the vicinity of Washington, D. C., the ragweeds regularly shed their first pollen about the middle of August. It may be considered that the earliest flowering plants bloom about this date each season because they react to a length of day somewhat less than that of the longest day, which is about 15 hours in this latitude. In other words, as soon as the decreasing length of day falls somewhat below 15 hours, a condition which obtains about July 1, the period of purely vegetative activity is checked, and the flowering phase of development is initiated. Should the seeds of such plants now be carried as far as northern Maine into a latitude of 46° to 47°, these plants would not experience a length of day falling below 15 hours in length, for which it is assumed they are best

[1] In this connection the tables showing the time of sunrise and sunset at 10-day intervals through the year for various latitudes in North America, as given in SMITHSONIAN CONTRIBUTIONS TO KNOWLEDGE, v. 21 (1876), p. 114–119, will be found very convenient for reference.

suited, until about August 1. In this latitude, then, provided other conditions did not intrude, flowering would be delayed until about August 1, and the chances of successfully maturing seed before killing frosts intervened would be greatly lessened. If the seed were carried still farther north, the plants might not blossom at all, owing to the fact that even the shortest days of the summer growing period would exceed those to which they were best suited in their normal habitat. Although in such instances these failures naturally would have been explained in the past on the basis of unfavorable temperature relations alone, it is obvious that length of day, primarily, is the limiting factor which has retarded the reproductive period so that unfavorable temperature relations have intervened to prevent the ripening of seed.

Although the Arctic summers are very short, plants have become successfully established under such conditions, largely by the development of specialized perennial types, which find the extremely long days favorable both to vegetative growth and to flower production. Although it has been usually considered that the purely Arctic forms are confined to Arctic conditions because of certain temperature requirements, etc., it is possible that length of day, hitherto overlooked as a factor in plant distribution, may have much to do with their restricted range apart from other factors of the environment.

In tropical regions it is probable that the success of many native plants is more or less closely dependent upon the conditions of equal or nearly equal days and nights which prevail there during the entire year. The varieties of bean coming from Peru and Bolivia appear to be of this type. It is evident that such plants, whose flowering conditions depend more or less closely upon a length of day little if at all exceeding 12 hours, can not attain the flowering stage attended by successful seed production in higher latitudes, at least during the summer season, which would necessarily be characterized by days in excess of 12 hours. It is indicated by the beans in question, however, that some plants of this class may grow and attain successful seed production under day lengths less than 12 hours. This being the case, such plants could at least extend their range beyond the Tropics in so far as the temperature conditions of the winter months in these latitudes were favorable to growth and reproduction.

In any study of the phenological aspects of different species of plants the fact stands out that certain plants bloom at definite seasons of the year. This is quite as marked in subtropical regions as in more northern regions having a definite summer growing season. In this connection it is probable that the relative lengths of the days and nights are of particular significance in many instances. The behavior of the composite *Mikania scandens*, as observed under specially controlled conditions and under winter conditions in the greenhouse, may be more critically considered in relation to its normal blooming season throughout its range.

This plant normally blooms from late July to middle or late September, indicating that blossoming becomes more or less inhibited as the autumnal equinox is passed in late September and the length of the day falls below 12 hours. In the greenhouse at Washington the short days of the winter, ranging around 9 to 10 hours in length, have completely inhibited the flowering phase of development of this plant. The shorter 7-hour daily exposures to light under controlled conditions have produced identical results. Thus it appears that the normal flowering period of *Mikania scandens* even in the warmer portions of its range should not occur much later in the season than the period when the days are not less than 12 hours in length. This seems to be the case in Florida, where the blooming season of Mikania is confined to August and September, as it is in much more northern portions of its range. Plants of this type, attaining their best development under daylight lengths of approximately 12 hours, should also find a more or less congenial environment under truly tropical conditions where the days are never much less than 12 hours in length. It is probable that in the Tropics, however, many plants of this type would not only become perennial in their aerial portions, but would also have a more or less continuous flowering period.

Since it has been shown that the stature of some plants increases in proportion to the length of the day to which the plants are exposed under experimental conditions, this factor should be expected to have some influence upon the stature of such plants in their normal habitat. In general, exceptional stature would be attained in those regions in which a long day period allowed the plants to attain their maximum vegetative expression before the shorter days intervened to initiate the reproductive period. This condition should hold true not only for different latitudes where a plant has an extensive northward and southward range but for different sowings in the same locality at successively later dates during the season. It is a matter of common observation that the rankest growing individuals among such weeds as the ragweed, pigweed (Amaranthus), lamb's quarters (Chenopodium), cocklebur (Xanthium), beggarticks (Bidens), other conditions being equal, are those which germinated earliest in the season, and consequently were afforded the longest favorable period of vegetative activity preceding the final flowering period. It is also a matter of common observation that all these weeds, when germinating very late in the summer and coming at once under the influence of the stimulus of the shortening days, blossom when very small, often at a height of only a few inches.

Many species of plants have an extensive northward and southward distribution. In these instances it may be that such species are capable of reacting successfully to a wide range of different lengths of day, or it is possible that the apparent adjustment to such a wide range of conditions may depend upon slightly different physiological requirements of different types which have been developed as a result of natural selections. It yet

remains to be seen whether those individuals of a given species which grow successfully in high latitudes have the same physiological requirements with respect to length of day as those growing quite as successfully near the equator. In any study of the behavior of plants introduced from other regions, with a view to determining certain economic qualities, it is evident that the factor of length of day must be taken into consideration as a matter likely to have great significance.

LENGTH OF DAY AS A FACTOR IN CROP YIELDS

From the facts which have been developed in this paper it would seem that the seasonal change in length of day is a hitherto unrecognized factor of the environment which must be taken into account when dealing with the problems in crop production. So far as is now known, the length of the day is the most potent factor in determining the relative proportions between the vegetative and the fruiting parts of many crop plants; and, in fact, as already pointed out, fruiting may be completely suppressed by a length of day either too long or too short. In some crop plants the vegetative parts alone are chiefly sought, while in others the fruit or seed only are wanted, and in still others maximum yields of both vegetative and reproductive parts are desired. It is apparent that the merits of different varieties or strains may depend largely on the relative length of day in which they are grown, and, therefore, the date of planting may easily become the decisive factor. These are matters of vital importance to the plant breeder and the agronomist. Obviously, a delay of even two or three weeks in seeding certain crops because of inclement weather conditions or other considerations may bring about misleading results. It is to be remembered, furthermore, that planting too early may be equally inadvisable, for crops requiring relatively short days for blossoming may thus come under the influence of short days in early spring, resulting in "premature" flowering and a restricted amount of growth. An impressive lesson as to the influence of length of day on the size attained by the plant before blossoming is seen in the relative heights of consecutive plantings of the Biloxi soybean, as shown in figure 3. For maximum yields of many crops it is essential that the date of planting be so regulated as to insure exposure of the plant to the proper length of day, due regard being had for the specific light requirements of each crop as well as for the relative values of the vegetative and fruiting portions of the plant.

RESULTS OBTAINED WITH ARTIFICIAL LIGHT USED TO INCREASE THE LENGTH OF THE DAILY ILLUMINATION PERIOD DURING THE SHORT DAYS OF WINTER

These results are of particular significance, since increasing the duration of the illumination period of the short winter day by the use of electric light of comparatively low intensity has consistently resulted in

initiating or inhibiting the reproductive or the vegetative phases of development, depending upon whether the plants employed normally require long or short days for these forms of expression. In these experiments a greenhouse 50 feet long, 20 feet wide, and 12 feet high to the ridge, with side walls of concrete 5 feet high to the eaves, was provided with 34 tungsten filament incandescent lights, each rated at 32 candlepower, evenly distributed beneath the glass roof. As a control, a similar greenhouse without artificial light was used. The long axis of these houses was on a north and south line. The temperature was approximately the same in the two greenhouses, ranging at night around 60° to 65° F. and 75° to 80° during the day. The unlighted greenhouse, however, tended to run two to three degrees higher than the illuminated house. Beginning on November 1 the electric lights were switched on at 4.30 p. m. and turned off at 12.30 a. m., this procedure being followed throughout the course of the experiments. Supplementing the natural length of the winter days with this 8-hour period of artificial illumination has given about 18 hours of continuous daily illumination, approaching in length the summer days of southern Alaska. Under these conditions the following results have been obtained:

A large clump of *Iris florentina* L., with all earth intact, was transplanted October 20, 1919, to each of the two greenhouses. The plants exposed to the long daily period of illumination began growing vigorously at once, soon attaining the normal size for this species, and produced blossoms on December 24 and December 30. The controls remained practically dormant and showed no tendency to blossom as late as February 12, 1920.

Seed of spinach (*Spinacea oleracea* L.), Bloomsdale Curley Savoy,[1] was sowed November 1, 1919, and came up in both houses on November 6. The plants in the control house, 20 to 25 in number, grew very slowly, producing low, compact, leafy growths or rosettes, and gave no evidence of blossoming as late as February 12. The plants in the lighted house elongated very rapidly, soon developing flower stalks, and all blossomed in the period between the dates December 8 and December 23. These have continued to elongate more or less, blossoming and shedding pollen continuously, thus becoming in effect "everblooming" plants.

Seed of cosmos (*Cosmos bipinnata* Cav.) was sowed November 1, 1919, and germinated in both houses November 5. In each greenhouse 40 to 45 plants were grown. The plants in the control house quickly flowered, and all blossomed in the period from December 22 to January 2. The plants in the lighted house grew well but remained in the strictly vegetative stage and were showing no indications of blossoming on February 12. On this date the control plants averaged 30 inches in height and the plants in the lighted house 60 inches.

[1] Horticultural variety.

Seed of radish (*Raphanus sativus* L.), Scarlet Globe,[1] was sowep November 1, 1919, coming up in both lighted and unlighted houses November 5. On February 12 the control plants, although more stocky and having larger roots, showed no indications of developing flower stems. In the lighted house, however, the plants had developed smaller roots, and flower buds were plainly in evidence, showing that the plants would soon blossom, as is their normal behavior in response to the long summer days out of doors.

Seedlings of the Maryland Mammoth variety of tobacco were transplanted to 12-quart iron pails on November 10, on which date they were placed in the control and the lighted houses. The control plants, six in number, exhibited the typical behavior of winter-grown Maryland Mammoth plants, all blossoming during the period from December 31 to January 8. The plants in the lighted house, six in number, behaved as typical summer-grown mammoths, becoming very compact, stout and leafy, with no indications of blossoming on February 12. On this date these plants had already produced many more leaves than the control plants.

Bulbs of *Freesia refracta* Klatt were placed in soil in 5-inch pots on July 11, 1919. Four pots of these plants were kept in the large dark house previously described from 4 p. m. to 9 a. m. daily from July 23 till November 15, when they were transferred to the greenhouses, two pots being placed in the control house and two in the lighted house. None of these plants when taken from the dark house on November 15 showed any indications of blossoming. Both lots began blossoming about December 27. In the control house, however, the plants produced many flower stalks and continued to blossom profusely for a long period. The plants in the lighted house, on the contrary, produced but few flower stalks and few blossoms and soon ceased blooming entirely.

Large, robust clumps of wild violets, of the species *Viola papilionacea* Pursh, were transplanted to pots and boxes and placed in the control and lighted houses on October 31, 1919. At the time these plants were removed from the field the abnormally warm autumn weather had forced them into bloom, and many purple, petaliferous blossoms were in evidence. As the winter days continued to shorten naturally in the control house, blossoming was suppressed and no new leaves were produced. These control plants appeared to be almost dormant, except for the production of numerous short, thickened stems which were crowded close to the ground among the old leaves. In the lighted house the production of the purple, petaliferous blossoms also ceased, but vegetative growth was initiated and new leaves appeared in great abundance. Coincident with this marked vegetative activity, the plants continuously produced fertile, cleistogamous flowers in great abundance. This furnishes another example of ever-blooming in response to a favorable length of day. In all respects this behavior of the violets in the lighted house

[1] Horticultural variety.

simulates the normal behavior of these plants out of doors under the influence of the long summer days.

Through the kindness of Dr. D. N. Shoemaker, several varieties of Lima beans (*Phaseolus lunatus* L.), F. S. P. I. No. 46153, from Chincha, Peru, and F. S. P. I. No. 46339, from Guayaquil, Eucador, were transplanted from the field to the greenhouse. Several plants of each of these varieties were transplanted into each house on October 18, 1919. Up to that time the plants had not flowered but gave evidence in the field of being extremely late varieties in this latitude. The control plants grew rather slowly but soon became markedly floriferous, setting pods freely. On the other hand, the plants in the artificially illuminated house produced an exceptionally rank growth of vines but did not flower.

Biloxi, Tokyo, Peking, and Mandarin varieties of soybeans were sowed November 1, 1919, and came up November 10 in both houses. In the control house the first blossoms appeared on the Biloxi and Mandarin about December 24, and on the Tokyo and Peking about December 18. In the artificially lighted house the early variety, Mandarin, blossomed on about the same date as in the control house; but under the longer light exposure the plants continued to grow vigorously, and only a very few blossoms appeared, suggesting a tendency toward gigantism. The few blossoms which formed, however, were normal and developed normal pods, while those on the control plants were cleistogamous and sterile. As late as February 12 the other three varieties showed no indications of blossoming. On that date all varieties were much taller in the electrically lighted house than in the control house.

Seed of Beggar-ticks (*Bidens frondosa* L.) were sowed in both houses on November 19, 1919, and came up in each on December 1. When the plants were very small they were transferred from the flats to 5-inch pots. The transplanting took place on December 19, and on January 12 all the control plants in the unlighted house, 8 or 10 in number, were showing tiny flower heads, although these had attained a height of only 1 to 2 inches. These flower heads came into expression as soon as the plants had developed the second pair of foliage leaves above the cotyledons. The plants in the lighted house continued to produce vegetative growth and gave no evidence of producing flower heads as late as February 12. Two of these plants which had attained a height of 8 to 9 inches in response to the lengthened period of illumination in the artificially lighted house were transferred to the control house on December 19. On January 12 both plants had produced flower heads in response to the naturally short winter days prevailing in this house and gave promise of blossoming in a short time. The sister plants remaining in the illuminated house continued to produce vegetative growth, with no evidence of blossoming.

Buckwheat (*Fagopyrum vulgare* Hill) was sowed November 1, 1919, and came up in both houses on November 7. In the control house 28

plants were grown, and 32 plants were grown in the illuminated house. The dates on which the first blossoms appeared on the control plants exposed to the short winter days extended over a range of only about a week—from December 4 to December 10, inclusive. On the other hand, the dates on which first blossoms appeared on the plants exposed to the artificially lengthened day extended over a period of about four weeks—from December 6 to January 2, inclusive. On February 12 the control plants averaged uniformly only 24 inches in height and had practically ceased growing and blooming. The plants in the artificially illuminated house, on the contrary, continued to grow vigorously and to flower freely, having attained an average height of 58 inches, some of the taller being more than 9 feet in height on February 12. These taller plants blossomed much later than the others and produced very few blossoms, thus showing a tendency to become giant forms in response to the artificially produced longer day. The ever-blooming tendency of the plants as a whole, however, was much more marked under the influence of the lengthened illumination period than in the control greenhouse. Again, although the control plants showed very uniform behavior in the range of their earliest blossoming, it is evident that the artificially lengthened period of illumination has in some manner led to a greatly extended range in the time of blossoming. Whether this really represents an unequal response of several more or less distinct, intermingled races to the artificially increased length of day or may be due in part to a more profound physiological variability which has been induced can not be determined until systematic selection and breeding studies have been carried on.

It will be evident that these data dealing with an artificially lengthened illumination period obtained by means of the electric light greatly strengthen the results of the experiments secured during the previous summer by artificially shortening the natural period of illumination through the use of dark houses. The results with the Maryland Mammoth variety of tobacco, the several soybean varieties in question, and the radish are of special significance since they were obtained by methods the direct converse of those used during the summer. Although the intensity of the electric light was undoubtedly far below that of normal sunlight, it was sufficient to initiate or to suppress the reproductive and vegetative activities of these three species as did the long days of the summer time. With respect to the ever-blooming behavior of certain of the plants under study, the results obtained indicate that this behavior is likely to follow when an approximately constant daily illumination period of a duration favorable to both growth and reproduction is maintained for a sufficient length of time. It thus seems possible that the comparatively uniform length of day prevailing in the Tropics accounts for the particular abundance of ever-bloomers in that region.

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IS THE RESPONSE TO DIFFERENCES IN THE LENGTH OF DAY A PRINCIPLE OF GENERAL APPLICABILITY IN BIOLOGY?

Experience has abundantly demonstrated the fact that the biologist who attempts to draw sweeping generalizations regarding responses of plants or animals as a whole to conditions of the environment is in serious danger of going astray, even though his observations be based on the behavior of relatively large numbers of species. With this fact clearly in mind, the following suggestions are put forward tentatively but as possibly being of sufficient interest to justify careful consideration on the part of biologists especially concerned in the fields touched upon. It has been clearly brought out in this paper that for a number of plant species the appropriate length of day acts, not merely as an accelerative, but rather as an initiative influence in bringing into expression the plant's potential capacity for sexual reproduction. Perhaps, as an equally satisfactory way of expressing the fact, it may be said that the length of the day exercises a truly determinative influence on plant growth as between the purely vegetative and the (sexually) reproductive forms of development. The response to length of day may be expected to hold for other species, although it would be premature at present to assert that all higher plants will be found to respond to this factor.

One is naturally inclined to inquire whether, also, the length of day is a controlling factor in sexual reproduction among the lower forms of plant life. The observed behavior of some of these lower forms certainly suggests that they come under the influence of the seasonal range in length of day. A single instance will suffice to illustrate the parallelism existing between the vegetative and the reproductive periods of activity, on the one hand, and the periodical change in the length of the day, on the other. Reference is made to the work of Lewis (*14*), in which it is shown that in certain species of red Algae there is a definite seasonal periodicity in the appearance of sexual and asexual forms. In brief, the July growth of these species consists primarily of tetrasporic or asexual individuals, while through August the growth is characterized by a predominance of sexual plants produced from the tetraspores of the July crop of plants. The carpospores of autumn become sporelings which persist through the winter and give rise to the tetrasporic plants of the early summer period. Should it be true that lower plants respond to differences in length of day as do some of the higher species it may be expected that various relationships between annual and perennial forms, differences in sensibility to relatively long and short days, and other facts which have been shown to apply to these higher species would likewise hold true for lower organisms. It is possible, even, that the seasonal activities of some of the parasitic microorganisms are the result of response to changes in day length.

As to animal life nothing definite can be said, but it may be found eventually that the animal organism is capable of responding to the

stimulus of certain day lengths. It has occurred to the writers that possibly the migration of birds furnishes an interesting illustration of this response. Direct response to a stimulus of this character would seem to be more nearly in line with modern teachings of biology than are theories which make it necessary to assume the operation of instinct or volition in some form as explaining the phenomena in question.

CONCLUSION

The results of the experiments which have been presented in this paper seem to make it plain that of the various factors of the environment which affect plant life the length of the day is unique in its action on sexual reproduction. Except under such extreme ranges as would be totally destructive or at least highly injurious to the general well-being of the plant, the result of differences in temperature, water supply, and light intensity, so far as concerns sexual reproduction, appears to be, at most, merely an accelerating or a retarding effect, as the case may be, while the seasonal length of day may induce definite expression, initiating the reproductive processes or inhibiting them, depending on whether this length of day happens to be favorable or unfavorable to the particular species. In broad terms, this action of the length of day may be tentatively formulated in the following principle: Sexual reproduction can be attained by the plant only when it is exposed to a specifically favorable length of day (the requirements in this particular varying widely with the species and variety), and exposure to a length of day unfavorable to reproduction but favorable to growth tends to produce gigantism or indefinite continuation of vegetative development, while exposure to a length of day favorable alike to sexual reproduction and to vegetative development extends the period of sexual reproduction and tends to induce the "ever-bearing" type of fruiting.

The term *photoperiod* is suggested to designate the favorable length of day for each organism, and *photoperiodism* is suggested to designate the response of organism to the relative length of day and night.

SUMMARY

(1) The relative length of the day is a factor of the first importance in the growth and development of plants, particularly with respect to sexual reproduction.

(2) In a number of species studied it has been found that normally the plant can attain the flowering and fruiting stages only when the length of day falls within certain limits, and, consequently, these stages of development ordinarily are reached only during certain seasons of the year. In this particular, some species and varieties respond to relatively long days, while others respond to short days, and still others are capable of responding to all lengths of the day which prevail in the latitude of Washington where the tests were made.

(3) In the absence of the favorable length of day for bringing into expression the reproductive processes in certain species, vegetative development may continue more or less indefinitely, thus leading to the phenomenon of gigantism. On the other hand, under the influence of a suitable length of day, precocious flowering and fruiting may be induced. Thus, certain varieties or species may act as early- or late maturing, depending simply on the length of day to which they happen to be exposed.

(4) Several species, when exposed to a length of day distinctly favorable to both growth and sexual reproduction, have shown a tendency to assume the "ever-blooming" or "ever-bearing" type of development—that is, the two processes of growth and reproduction have tended to proceed hand in hand for an indefinite period.

(5) The relationships existing between annuals, biennials, and perennials, as such, are dependent in large measure on responses to the prevailing seasonal range in length of day. In many species the annual cycle of events is governed primarily by the seasonal change in length of day, and the retarding or more or less injurious and destructive effects of winter temperatures are largely incidental rather than fundamental. Hence, by artificial regulation of the length of the daily exposure to light it has been found that in certain species the normal yearly cycle of the plant's activities can be greatly shortened in point of time, or, on the other hand, it may be lengthened almost indefinitely. In certain cases, annuals may complete two cycles of alternate vegetative and reproductive activity in a single season under the influence of a suitable length of the daily exposure to light. Similarly, under certain light exposures some annuals behave like nonflowering perennials.

(6) In all species thus far studied the rate of growth is directly proportional to the length of the daily exposure to light.

(7) Although the length of the daily exposure to light may exert a controlling influence on the attainment of the reproductive stage, experiments reported in this paper indicate that light intensity, within the range from full normal sunlight to a third or a fourth of the normal, and even much less, is not a factor of importance. It follows that the total quantity of solar radiation received by the plant daily during the summer season, within the range above indicated, is of little importance directly so far as concerns the attainment of the flowering stage.

(8) In extensive tests with soybeans, variations in the water supply ranging from optimum to a condition of drought sufficient to induce temporary wilting daily and to cause severe stunting of the plants were entirely without effect on the date of flowering, although in some cases drought seemed to hasten somewhat the final maturation of the seed. Similarly, differences in light intensity, in combination with differences in water supply, failed to change the date of flowering in soybeans.

(9) The seasonal range in the length of the day is an important factor in the natural distribution of plants.

(10) The interrelationships between the length of day and the prevailing temperatures of the winter season largely control successful reproduction in many species and their ability to survive in given regions.

(11) The relation between the length of the day and the time of flowering becomes of great importance in crop yields in many instances and in such cases brings to the forefront the necessity for seeding at the proper time.

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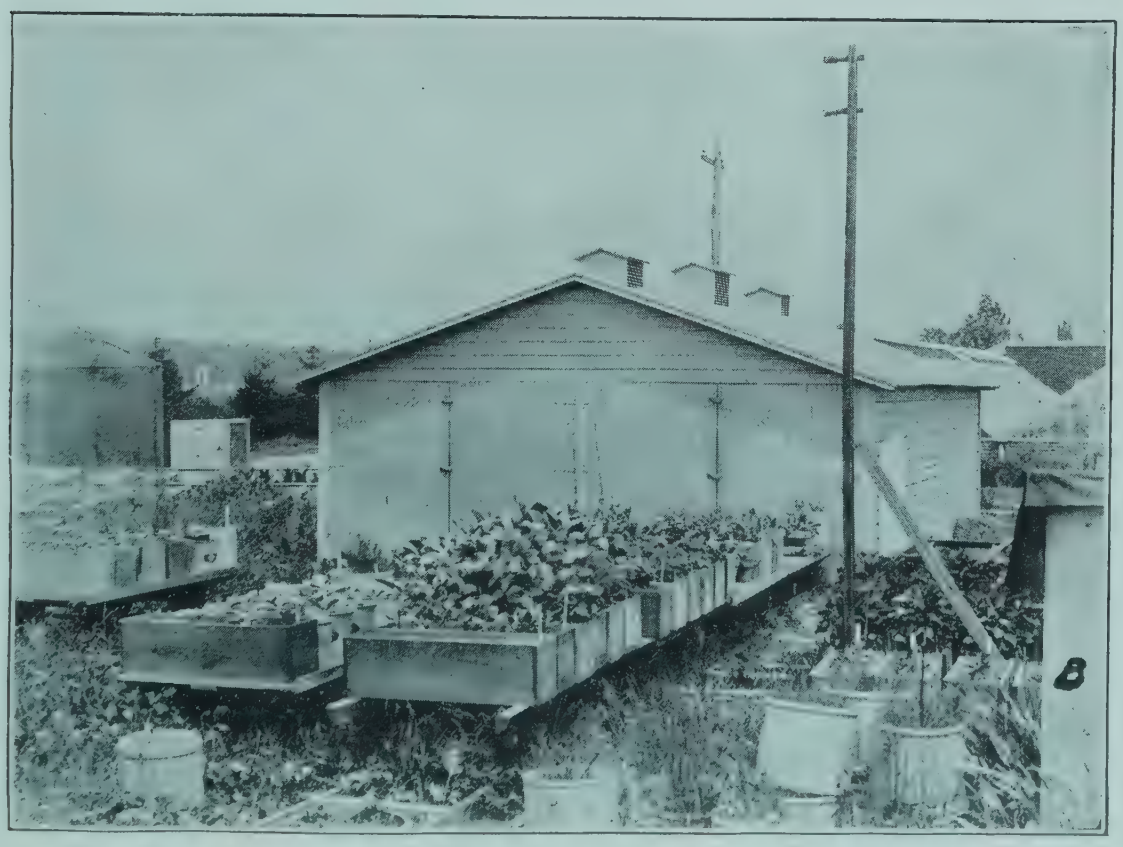
PLATE 64

A.—Small dark chamber used in the 1918 experiments.

B.—Larger dark house used in the 1919 tests. Trucks and steel tracks used in moving the test plants into and out of the dark house.

A


B

A

B

PLATE 65

A.—Peking soybeans exposed to the light for 7 hours daily. Note the abundance of full-sized green seed pods. Photographed July 9, 1919.

B.—Control planting of Peking soybeans exposed to the light for the whole day—that is, kept out of doors day and night. No blossoms had appeared when photographed July 9.

PLATE 66

A.—Peking soybeans exposed to the light for 7½ hours daily, beginning with the blossoming period. When photographed September 13, 1919, the seed pods had fully matured and were ready for harvest.

B.—Control planting of Peking soybeans kept out of doors throughout the test. When photographed September 13 the seed pods were still green and the foliage was just beginning to yellow slightly.

A.

B

A

B

PLATE 67

A.—Biloxi soybeans exposed to the light for 7 hours daily. When photographed August 15, 1919, all seed pods were mature and dry and the leaves were falling.

B.—Control planting of Biloxi soybeans kept out of doors during the test. When photographed August 15 there were no indications of blossoming.

PLATE 68

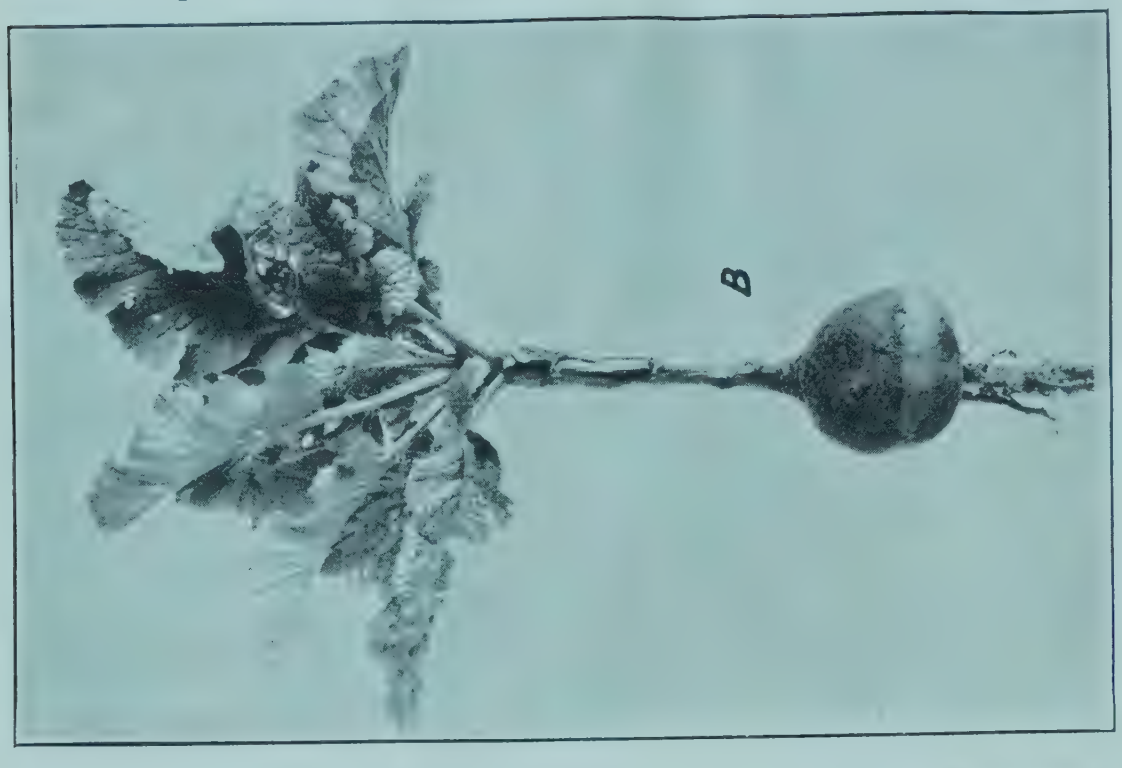
Biloxi soybeans:

A.—Plants in box on left exposed to the light for 5 hours daily. Those in box on right kept out of doors throughout the experiment. When photographed August 15, 1919, the plants on left contained fully matured seed pods and leaves were yellowing, while plants on right had not blossomed.

B.—Plants in box on left exposed to light daily for 7 hours; those on right exposed 12 hours daily. While both lots blossomed and fruited promptly, the plants under the longer light exposure grew much larger than those under the shorter exposure. Photographed August 19.

A

B


B

A

PLATE 69

A.—Biloxi soybeans. Plants in box on right exposed to light from daylight to 10 a. m. and from 2 p. m. to dark, a total of 9 to 10 hours daily. Plants in box on left exposed to light from 6 a. m. to 6 p. m., or 12 hours daily. Note marked difference in effectiveness of the two exposures in hastening fruiting and maturation. Photographed September 8, 1919.

B.—Radish plant in which the seed stalk was transformed into a vegetative shoot through the influence of the decreasing length of day. Photographed October 12.

PLATE 70

A.—Maryland Mammoth tobacco in 8-inch pots exposed to light from 9 a. m. to 4 p. m. daily. Seed pods full-grown when photographed August 15, 1919.

B.—Control series of Maryland Mammoth plants kept out of doors. No signs of flowering when photographed August 15.

A

B

A

B

PLATE 71

A.—Maryland Mammoth tobacco in 12-quart buckets exposed to light from 6 a. m. to 6 p. m., or 12 hours daily. Flower heads forming but no open blossoms present when photographed August 19, 1919.

B.—Maryland Mammoth tobacco in 12-quart buckets exposed to light from 9 a. m. to 4 p. m., or 7 hours daily. Seed pods formed when photographed August 19.

PLATE 72

A.—Control series of Maryland Mammoth tobacco in 12-quart buckets left out of doors during the experiment. Flower heads just beginning to show when photographed August 19, 1919.

B.—*Aster linariifolius* L. Plants in box on left exposed to light from 9 a. m. to 4 p. m. daily. In full bloom when photographed June 24. Plants in box on right left out of doors during the test. Showed no indications of flower heads when photographed June 24.

A

B

A

B

PLATE 73

A.—*Phaseolus vulgaris* from Peru and Bolivia exposed to light from 9 a. m. to 4 p. m. Contained full-grown seed pods when photographed August 15, 1919.

B.—Control series of Phaseolus left out of doors during the experiment. Showed no indications of flowering when photographed August 15.

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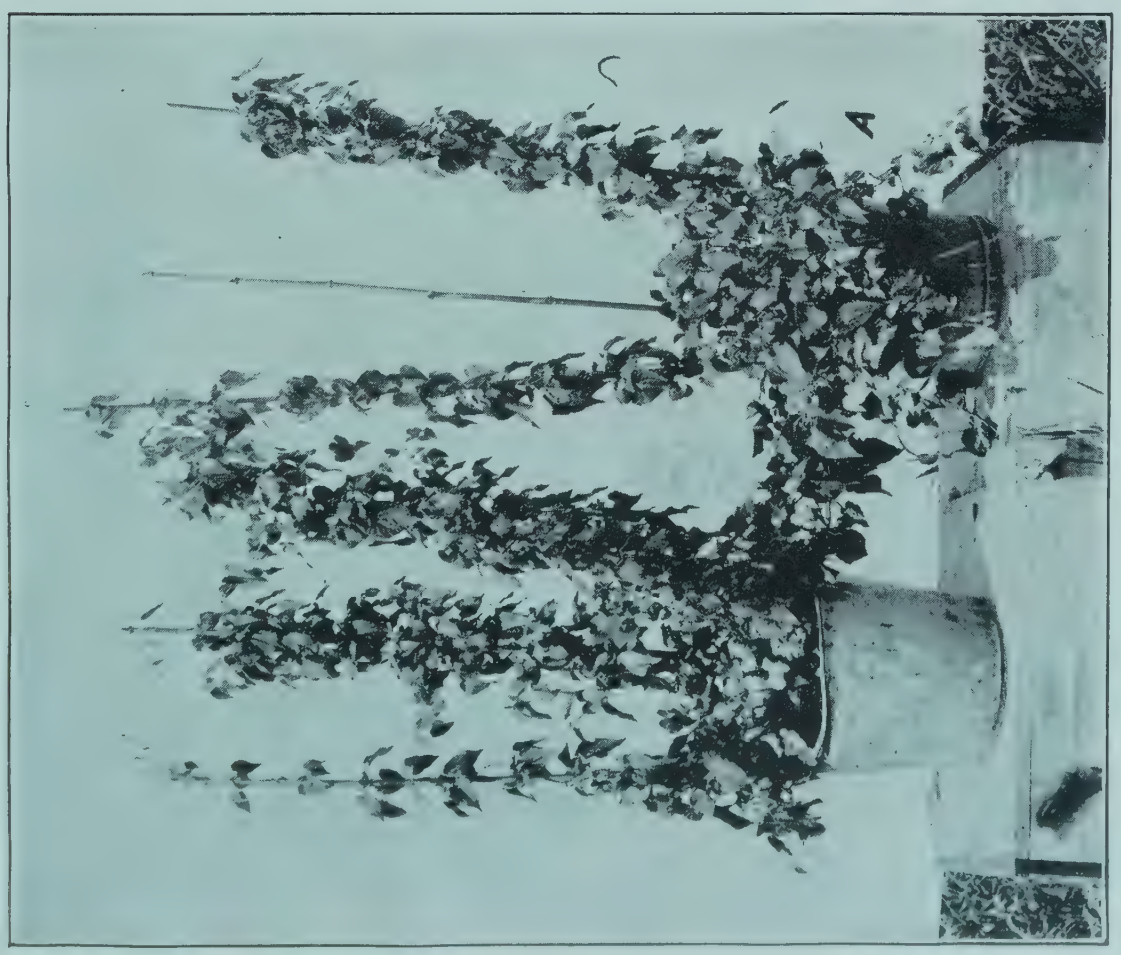
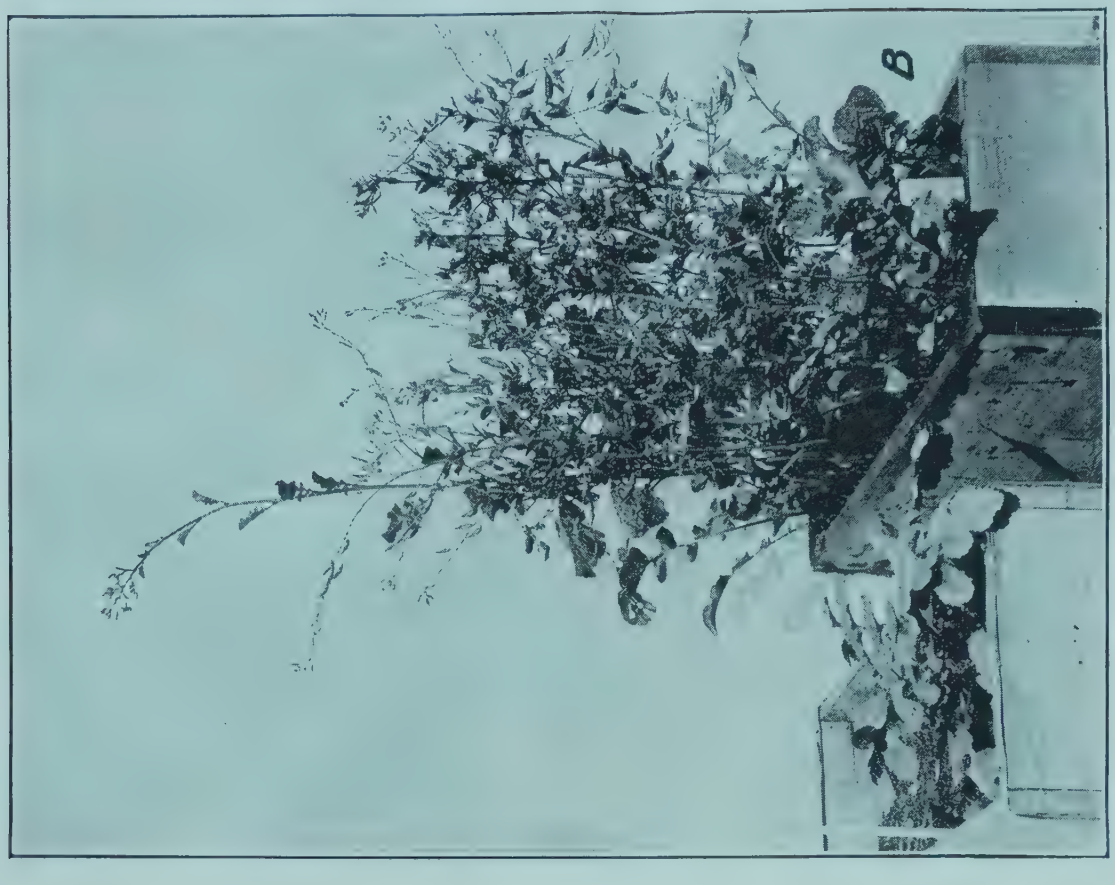
PLATE 74

A.—*Mikania scandens* L. exposed to light from 9 a. m. to 4 p. m. daily. Showed no indications of flowering when photographed August 15, 1919.

B.—Control plants of Mikania left out of doors during the test. Blossoming profusely when photographed August 15.

B


C
A


B

A

PLATE 75

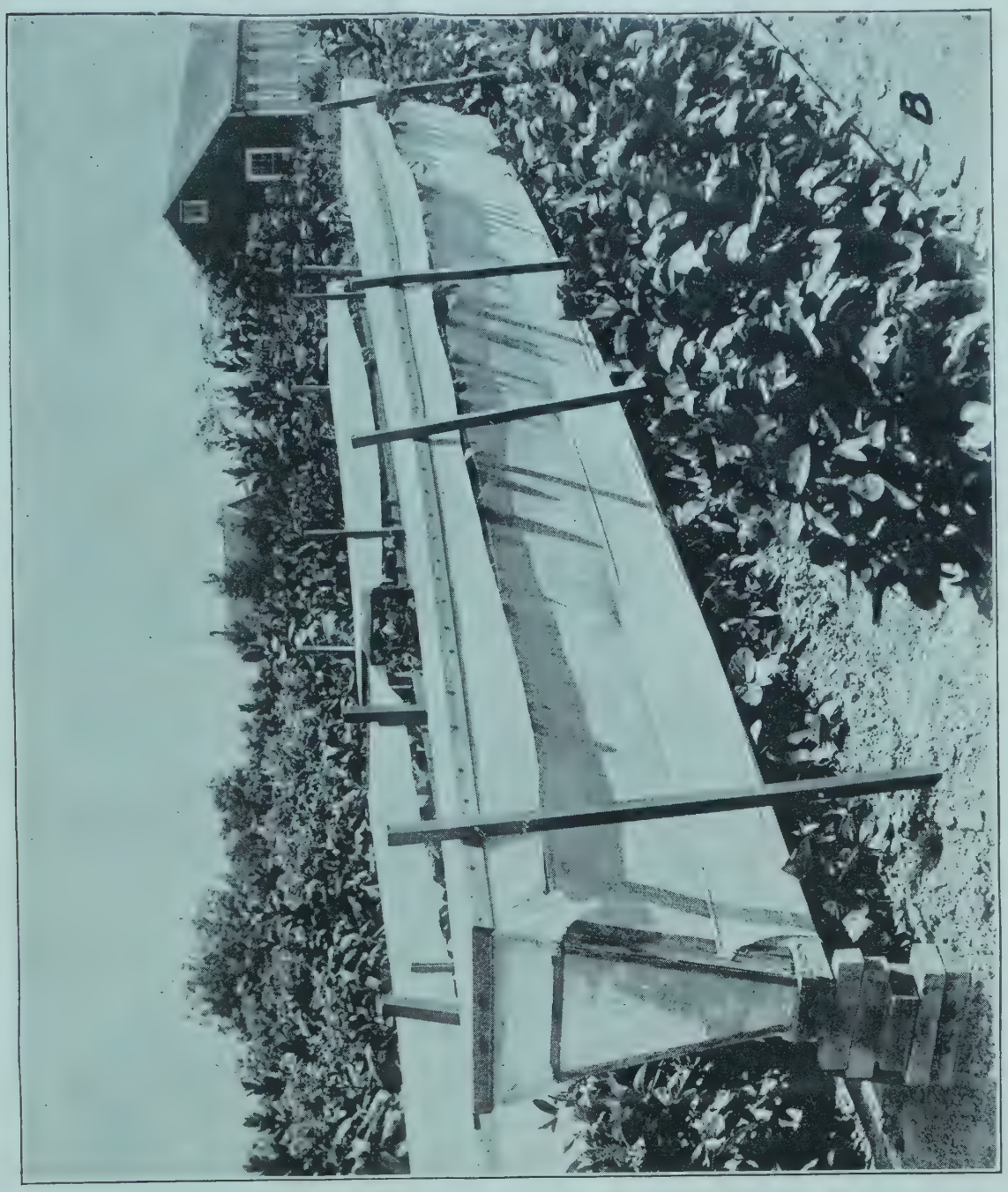
A.—Ragweed. Plants on left exposed to light from 9 a. m. to 4 p. m. daily. Pollen shedding freely from the staminate spikes when photographed July 9, 1919. Plants on right left out of doors as controls. Showed no signs of flowering when photographed.

B.—Radish. Plants in box on left exposed to light from 9 a. m. to 4 p. m. daily. No indications of seed stalks when photographed August 19. Plants in box on right left out of doors during the test. Bore an abundance of full-grown seed pods when photographed.

PLATE 76

A.—Portion of stem of Maryland Mammoth tobacco plant, showing the sharp delimitation of dying back of the original growth as controlled by the appearance of new shoots.

B.—Triangular type of shade with cheesecloth covering, used in the 1916 test with soybeans.


B

A

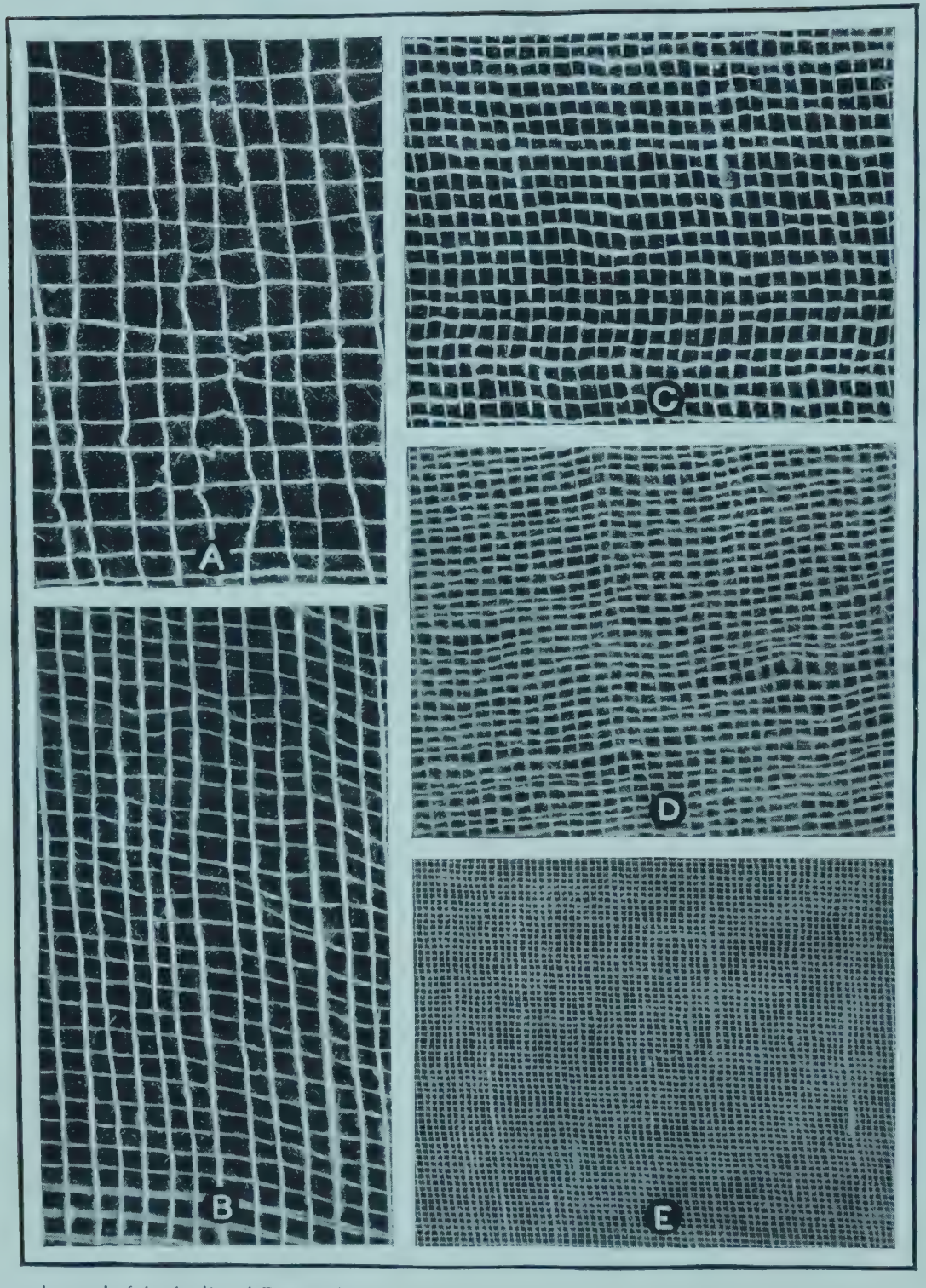

A
B
C
D
E

PLATE 77

A.—Shade cloth, 6 by 6 mesh. Natural size.
B.—Shade cloth, 8 by 10 mesh. Natural size.
C.—Shade cloth, 12 by 12 mesh. Natural size.
D.—Shade cloth, 12 by 20 mesh. Natural size.
E.—Standard cheesecloth used in 1916 experiments. Natural size.

PLATE 78

A.—Soybeans growing in box set in soil (covers removed), shaded with 12 by 12 mesh netting. Soil kept relatively wet from germination to maturity.

B.—Series of consecutive plantings of soybeans in the field during the summer. All planting shad flowered when photographed September 8, 1919. Note (from left to right) the rapid decrease in height of plants at the time of flowering as the dates of plantings become later and later.

A

B

A

B

PLATE 79

A.—Soybeans growing in four boxes set in the soil and provided with removable covers. To each box different measured quantities of water were added. In addition to the Peking seen in the farther halves of the boxes, an earlier variety, known as Little Brown, was grown in each box.

B.—Carrots. Plants in box on left exposed to light from 9 a. m. to 4 p. m. daily. Had made but little growth of top or root when photographed August 19, 1919. Plants in box on right left out of doors as controls. Had produced much larger tops and roots when photographed.

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